TABLE III

Varicose veins without ulceration

Patient	Sex	Age	Amount of edema per 100 cc leg‡	Blood oxygen before and after sitting	Blood oxygen at saturation	Oxygen saturation	CO_2 volume	Oxygen tension	Comment (Patients sitting unless otherwise specified)
		years	*cc*	*cc per 100 cc*	*cc per 100 cc*	*per cent*	*per cent*	*mm Hg*	
M E W	F	42	4 4	13 6 13 3	22 0	60 5	45 0 47 8	46 0 37 7	Feet swollen at night Left and short saphenous affected
M F	M	63	4 9	11 0 9 6	21 0 22 9	52 5 42 0	61 5 57 3	30 4 24 0	Hypertension Saphenous affected Had ulcers previous
W L	M	34	4 1	19 4 12 6	23 4 24 5	83 0 51 5	49 0 52 0	48 0 28 0	Saphenous vein only affected
M M L	F	38	7 0	13 0 14 0	18 4 19 1	70 5 73 0	50 0 47 0	38 0 39 0	History of phlebitis Feet swell towards night Saphenous and communicating veins incompetent
M C	F	49	8 1*	15 8 10 4	19 3 19 8	82 0 52 2	49 0 49 0	46 8 27 5	Previous ulcer Saphenous and communicating veins incompetent
C J B	M	47	7 6*	14 8 12 4	19 6 21 1	75 5 58 8	52 5 51 5	42 0 31 5	Saphenous and communicating veins incompetent
W J H	M	51	(5 4)†	11 5 8 6	18 1 20 5	63 5 42 0	59 0 58 0	35 2 24 2	Saphenous only affected (Standing)

* Pitting edema
† Not included in the mean because subject was standing
‡ The standard deviation of the mean for the edema fluid is 6 0±0 7

be increased only slightly above normal, unless the valves of the communicating veins are incompetent When this is the case the edema formation is greatly increased

It has already been pointed out that we were unable to demonstrate any truly significant differences as far as oxygen tension was concerned in the three groups normal, simple varicose, and varicose veins with ulceration Furthermore, we have been unable to demonstrate any significant difference in oxygen tension when the data are divided on the basis of competent or incompetent communicating veins Neither were we able to correlate significantly the oxygen tension with edema formation in any of these cases

DISCUSSION

The results demonstrate in individuals having simple varicosities of the great saphenous system of veins, that the rate of edema formation in the dependent leg is slightly but definitely increased over the rate found under the same conditions in normal subjects Furthermore, when the valves of the communicating veins are incompetent much greater tendency to edema formation exist than is the case when these valves are normal

According to Blalock's data and our data, diminished oxygen tension of the blood is not factor in either this phenomenon or in the development of varicose ulcers These oxygen studies are concerned of course with venous, not capillary blood As already pointed out, this may not give an index of the oxygen tension in a particular part of the capillary bed It is possible that the blood may be shunted around the edematous regions where the tissue tension is relatively high, the major portion of the blood tending to flow through less obstructed channels, or the blood may be diverted directly from arteriole to venule avoiding the capillary bed In any case, the venous blood may not give a fair representation of the oxygen tension in certain parts of the capillary system Studies of the saphenous blood offer no explanation of the increased tendency to edema formation described above

Failure of blood analysis to explain the finding

TABLE IV

Varicose veins with ulceration

Patient	Sex	Age	Amount of edema per 100 cc. leg‡	Blood oxygen before and after sitting	Blood oxygen at saturation	Oxygen saturation	CO_2 volume	Oxygen tension	Comment. (Patients sitting unless otherwise specified)
		years	*cc.*	*cc. per 100 cc.*	*cc. per 100 cc*	*per cent*	*per cent*	*mm Hg*	
M K.	M	48	*Before test						
			5 6	15 1	20 7	72 8	50 6	39 8	Ankles swell. Saphenous and communicating veins incompetent Ulcer at ankle
				13 2	22 0	59.5	51.5	32 0	
B M	F	46	*Before test						
			7 4	9.3	18 1	51 0	56 0	28 2	Saphenous and communicating veins affected
				9.3	19.3	47.5	54 0	26.2	
E. M	F	45	5 4	12 9	17 2	75 0	36 0	37 6	Ulcer 2 cm diameter Communicating and saphenous veins incompetent
				5 6	19 6	28 6			
W K.	M	26	(5.2)†	18 2	21.5	85 0	47 1	49 6	Probably arteriovenous anastomosis. Therefore not used in average of incompetent communicating veins Communicating veins incompetent
				19.3	22 4	86 5	44.5	51 0	
E. C. S	F	37	5 4	14 7	21 9	67 0	51 0	36 0	Communicating veins incompetent. Preulcerous condition
				11 7	22.2	53 0	48 0	27 8	
B E F	F	42	8 5*	13 5	21 0	64 0	47 0	33 0	Communicating veins incompetent
				10.3	21 8	47.5	47 0	24 8	

* Pitting edema.
† Not included in the mean because of question of arteriovenous anastomosis.
‡ The standard deviation of the mean for the edema fluid is 6.5±0 6

raises several questions First, why should incompetence of the valves of the communicating ins be associated with increased edema formaon? Two answers to this question are possible. ie valves of the communicating veins are found competent in most cases following deep phlebitis s a consequence of this condition it is probably ie that the valves of the *deep* veins are also incompetent following their recanalization (6) If e valves of the deep veins are incompetent these veins of the lower leg would be subjected to sustained high pressures These pressures during relaxation of the leg muscles would be hydrostatic, that is, dependent approximately upon the weight of the column of blood from the heart level to the level of the lower leg

At all times, with the patient in the upright position the pressure in the veins would then be far above the colloid osmotic pressure of the blood even during walking (See Beecher, Field, and Krogh (7)) The condition in the extensive deep system of veins would be comparable to that described by Beecher (8) for simple varicosities of the superficial veins Sustained high pressure in the superficial veins alone was not found associated with gross edema formation, presumably because the lymphatics were adequate to drain the relatively limited tissues drained by these veins But now, if the deep system of veins is exposed to a sustained high venous pressure this should greatly increase the quantity of tissue fluid formed during relaxation of muscles (According to Wells (9), the intramuscular pressures during contraction may be great enough to prevent this filtration) With incompetent valves of the communicating veins, this high pressure would be transmitted to the superficial system where presumably the valves of the saphenous system would also be inadequate and the pressure there already high It seems reasonable to suppose with *sustained* high pressures throughout the venous system of the leg that a greater quantity of tissue

fluid would be formed than when the superficial system alone was involved, and it is not surprising that the lymphatics are inadequate to transport away this greatly increased accumulation of tissue fluid Edema develops

Presumably, if these patients sat absolutely still with legs completely relaxed at all times the rate of formation of tissue fluid would be equally great in subjects with normal veins and those with incompetent valves, insofar as a sustained venous pressure is a factor in the development of edema Practically, these patients were not trained subjects and evidently moved around enough to prevent sustained high venous pressures from developing when the venous valves were competent In the ordinary conditions of life, of course, the difference between the normals and the group with inadequate venous valves would be exaggerated, and one would expect to find relatively far more edema fluid formed in the latter group

Another answer to the question raised above might be that the phlebitis which damaged the valves of the communicating veins may have damaged the lymphatic drainage system as well Drinker (10) has suggested that the lymphatics may be " varicose " Impairment of the lymphatic apparatus occurring together with the increased production of tissue fluid postulated above would be very effective in producing edema It seems likely that in certain cases both of these effects operate to produce edema A further condition tending to produce stagnation of lymph would be the limitation of movement when the edema became great enough to produce discomfort

Inadequacy of the lymphatic system of the extremity, from whatever cause, would be followed by edema of the lower leg and the preparation of a fertile field for the development of infection as Drinker's work (11) has shown Possibly the patient with incompetent valves of the communicating veins should be considered to be in a prevaricose ulcer condition The development of low grade infection is of considerable importance in the production and extension of varicose ulcers The rôle of fungus infection here has been inadequately studied to date

SUMMARY

We have measured the oxygen tension of the blood found in varices of the great saphenous system with and without ulceration Our data confirm Blalock in that we did not find low oxygen tensions to be present in these cases It is pointed out that study of saphenous blood may not provide a true index of the oxygen tension in certain parts of the capillary bed

The volume of edema fluid formed in three classes of subjects has been measured in the lower leg during a sitting period normals, cases with simple varicosities, and cases in which the varicosities were complicated by ulcer formation

A barely significant increase above normal occurs in the formation of tissue fluid when simple varicosities of the great saphenous system are present No significant increase in the formation of edema fluid was found in the patients having ulceration with their varicose veins over the varicose group without ulceration When a distinction was made between subjects with normal or with incompetent valves of the communicating veins a highly significant increase in tissue fluid formation was found in the latter group Possible reasons for this are discussed

CONCLUSIONS

1 Edema develops more readily in the legs of patients with varicosities than in normal individuals This tendency is greatly increased when the valves of the communicating veins are incompetent

2 Studies of the saphenous blood oxygen offer no explanation of this

BIBLIOGRAPHY

1 deTakáts, G, Quint, H, Tillotson, B I, and Crittenden, P J, The impairment of circulation in the varicose extremity Arch Surg, 1929, 18, 671

2 Blalock, A, Oxygen content of blood in patients with varicose veins Arch Surg, 1929, 19, 898

3 Van Slyke, D D, and Neill, J M, The determination of gases in blood and other solutions by vacuum extraction and manometric measurment. I J Biol Chem, 1924, 61, 523

4 Henderson, L. J, Bock, A V, Field, H, Jr, and Stoddard, J L, Blood as a physiocochemical system II J Biol Chem, 1924, 59, 379

5 Drury, A N, and Jones, N W, Observations upon the rate at which oedema forms when the veins of the human limb are congested. Heart, 1927, 14, 55

6 Edwards, E A, and Edwards, J E., The effect of

thrombophlebitis on the venous valve. Surg., Gynec. and Obst., 1937, 65, 310
7 Beecher, H K., Field, M. E., and Krogh A., The effect of walking on the venous pressure at the ankle. Skandinav Arch. f Physiol., 1936 73, 133
8. Beecher, H K., Adjustment of the flow of tissue fluid in the presence of localized, sustained high venous pressure as found with varices of the great saphenous system during walking J Clin. Invest. 1937, 16, 733
9 Wells H. S., Personal communication. 1938.
10 Drinker, C. K., Personal communication. 1938.
11 Drinker C. K., Field, M. E., Ward H. K. and Lyons, C., Increased susceptibility to local infection following blockage of lymph drainage. Am. J Physiol., 1935, 112, 74

The Journal of Clinical Investiga

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTI(

EDITORIAL BOARD

RANDOLPH WEST, M D, EDITOR-IN-CHIEF
Presbyterian Hospital, New York, N Y

FRANCIS G BLAKE, M D, *New Haven, Conn*
HERRMAN L BLUMGART, M D, *Boston, Mass*
JAMES L GAMBLE, M D, *Brookline, Mass*
ROBERT F LOEB, M D, *New York, N Y*
WILLIAM S MCCANN, M D, *Roc*
C PHILLIP MILLER, M D, *Chicag*
JOHN P PETERS, M D, *New Hav*
THOMAS M RIVERS, M D, *New*
FRANK N WILSON, M D, *Ann Arbor, Mich*

The Editorial Committee of the American Society for Clinical Investigatio

DAVID P BARR, M D, *St Louis, Mo*
WALTER BAUER, M D, *Boston, Mass*
ARTHUR L BLOOMFIELD, M D, *San Francisco, Calif*
ARLIE V BOCK, M D, *Boston, Mass*
C. SIDNEY BURWELL, M D, *Boston, Mass*
THOMAS FRANCIS, JR, M D, *New York, N Y*
GEORGE R. HERRMANN, M D, *Galveston, Texas*
L. EMMETT HOLT, JR., M.D, *Bal*
CHESTER S KEEFER, M D, *Bosto*
ROBERT F LOEB, M D, *New York*
EDWARD H MASON, M D, *Montr*
HUGH J MORGAN, M D, *Nashvil*
DAVID T SMITH, M.D, *Durham,*
FRED M SMITH, M D, *Iowa City*
ISAAC STARR, M.D, *Philadelphia, Pa*

THE JOURNAL OF CLINICAL INVESTIGATION is designed for the publication of original investigations dealing with or bearing on the problems of disease in man Each volume will consist of 6 bimonthly issues appearing throughout the calendar year

Contributions should be sent to the Editor-in-Chief, Dr Randolph West, Presbyterian Hospital, 622 West 168th Street, New York, N Y. Authors may purchase reprints at scheduled prices Further information regarding the preparation of manuscripts may be obtained from members of the Editorial Board of the Journal or of the Editorial Committee of the American Society for Clinical Investigation

The subscription price is $10 per volume of 6 bimonthly issues representing the issues of a calendar year, foreign countries $10 50 Single copies sold when availab tions are net postpaid Remitta made by draft, check or postal payable to THE JOURNAL OF VESTIGATION, Prince and Lem caster, Pa, or 654 Madison Av Claims for copies lost in the received within thirty days (don days (foreign), of the date of is of address must be received at l in advance of issue.

Correspondence concerning ters should be addressed to TH CLINICAL INVESTIGATION, Prin Sts, Lancaster, Pa., or 654 M New York

Entered as second-class matter September 16, 1931, at the Post Office at Lancaster, Pa, und March 3, 1879

Copyright, 1938, American Society for Clinical Investigation

PRESIDENT'S ADDRESS

CLINICAL EPIDEMIOLOGY[1]

JOHN R. PAUL

om the Department of Internal Medicine Yale University School of Medicine New Haven)

mpt to predict some of the trends Clinical Investigation may proceed in decades, the subject of Preventive urally arises as a field for these ac- term, Clinical Investigation in Pre- icine, is cumbersome and so I will In fact even the term, Preventive never seemed ideal It implies a h in the way of Propaganda. It pre- existence of a so-called sister science, dicine, and both sciences are com- os too definitely to a therapeutic pro- al Investigation in Epidemiology is ie purposes at hand, Clinical Epi- best, and really what I mean In ie name I would like to propose for e, a new discipline in which this So- ike an important part It is a science ith circumstances, whether they are ' or "organic," under which human one to develop It is a science con- the ecology of human disease. But in that, for any science worthy to be he name *Clinical*, should involve some e interpretation of the circumstances : deals It must face the question of well as "how" Clinical Epidemi- therefore, from the orthodox science ogy both in its aim, and its locale, as ie orthodox epidemiologist must of l dispassionately with large groups of s the multiplication of observations im his results The clinical epidemi- ie other hand, must of necessity deal groups of people, people whom he nd groups no larger than a family or inity The restriction of the size of sts on the fact that clinical judgment plied wholesale, without the risk of ead too thinly to be effective. Fortunately or unfortunately the amount of personal attention requisite for the exercise of clinical judgment is set by physiological limits which most of us cannot exceed. The clinical epidemiologist, therefore, can dispense just so much of this attention power at one time He starts with a sick individual and cautiously branches out into the setting where that individual became sick,—the home, —the family, and the workshop He is anxious to analyze the intimate details under which his patient became ill He is also anxious to search for other members of the patient's family, or community group who are actually, or potentially ill It is his aim to thus place his patient in the pattern in which he belongs, rather than to regard him as a lone sick man who has suddenly popped out of a healthy setting, and it is also his aim to bring his judgment to bear upon the *situation*, as well as on the patient.

Obviously there is nothing new to the family doctor about this concept of Medicine. It is the heart and soul of family practice and probably has been, as long as family practice has existed. But now that the emphasis, for this Society at least, has shifted away from the home and into the Hospital and Dispensary, clinical epidemiology will be practiced only if we take thought about it It is a foreign concept for most intramural clinical investigators whose contact with the actual circumstances under which their patients became ill may be limited to a page in the hospital history, or a supplementary talk with the social worker

To give a single but well known example of work in clinical epidemiology which has been accomplished in well known Institutions in this country, I will name the studies of Dr Opie and his coworkers on the spread of tuberculosis through families As a contribution to the field of tuberculosis, and also to other infectious diseases this work speaks for itself But the approach is not limited to Infectious Diseases It is being used by Dr Canby Robinson in the study of circumstances which are prone to give rise to a variety of types of illness which bring patients to

[1] President s Address before the American inical Investigation at its Thirtieth Annual it Atlantic City, N J., May 2 1938.

the Dispensary of the Johns Hopkins Hospital Clinical epidemiology is also something more than family visits As an example of another direction, and a most important direction it has taken, are the recent investigations concerned with the pathogenesis of pernicious anemia, and of nutritional deficiencies In these fields members of this Society have played no small part,—and, as such, the Society may also be said to have already had some share

The crux of these investigations in the various fields just mentioned lies not only in the discovery of new intrinsic or extrinsic factors, which may be found either indoors or outdoors, but in the discovery of new concepts The concept of certain new etiological forces which lie back of those which were once thought to be basic, such as for instance, the factors which lie back of the pneumococcus as a cause of pneumonia This is all so obvious that it hardly seems worth mentioning and yet a dominant thing about some of our present notions of causative factors is that unless they fit into a modern pattern of our own liking they are apt to be overlooked Of late years conservative opinion does not allow anything to be really considered as "etiology," unless we can succeed in getting it into a test tube, unless we can precipitate it,—unless we can crystallize it as it were This is due of course to our current methodology which has, perhaps, become more of a religion than most of us realize I think it may have led to a slightly narrow interpretation of clinical investigation on our part, for clinical investigation certainly should be given the opportunity to spread itself up into philosophy, if it will, as well as down into the basic sciences

Now this is not a plea for more papers describing philosophical concepts of epidemiology, for if they are really important they will find their way into our programs of their own accord without having to be plead for I only say that we ought not to be frightened by them We ought not to be frightened by the word clinical investigation in the field of Public Health, or clinical investigation outside the Hospital For, if we are frightened, then it may be true what our critics say, that we have become so attached to our own pet methods and points of view that we have drifted away from

But there is still another aspect to Clinical demiology which deals with the meaning of ease For instance, we may now have to dis smoke screen that the folklore of both Prevei Medicine and Curative Medicine has thrown which consist in a sort of censorship about meaning of disease, in which there are at least assumptions These are (A) that all disea bad and hence all attempts to prevent it, or cu are good, regardless of its cause or the condi under which it arises, and (B) that disea something which an unkind fate has put upon in other words disease is not of our own ma but it comes from elsewhere It is always "French disease" To turn the spotlight of vestigation upon these assumptions is the duty of the clinical epidemiologist It invo a certain amount of risk,—the risk of trifling religious tenets, and as such of being anti-so It might be anti-social if we found, for insta that all disease is not necessarily bad, but th wise Providence inflicts some one with arte sclerosis or even tuberculosis as a just reward his "bad living", or that children's diseases rained upon us as a means of furnishing us only with specific immunity, but who knows, much nonspecific immunity too, which may b inestimable value to us in adult life It m also be something of a betrayal of our clan if found that a good deal of illness may be lai our own feet, that is, illnesss caused by "ul modern therapeutics," *viz*, the creation of validism through overzealous treatment,—thro meddlesome treatment, and through the wl wretched system of abused sick benefits to wl we meekly bow our heads Although such fu tional causes of invalidism as these cannot be easily put into a test tube, and cannot be prec tated or crystallized they are powerful etiolog factors, intrinsic in our modern civilization, responsible for a good deal of *preventable illn* Strangely enough they have not yet been regar (by this Society at least) as a particular legitin

field for clinical investigation
something like bubonic plague
can conveniently be put on th
have followed too much in the

If these fields are eventual

were subsequently operated on Cases VII and VIII had definite hyperplasia histologically Case X was a doubtful lymphadenoid goiter A study of Figure 3 will show the total lack of relationship between a raised metabolism in hyperthyroidism and mobilization of bone salt as evidenced by the state of the calcium and phosphorus balance

Of the twelve cases only one had a negative calcium balance, and this case was having the first course of radiation at the time of the experimental period

The result of the studies on calcium and phosphorus balance on these cases indicates that thyroxin cannot be the cause of the mobilization of bone salt in hyperthyroidism

Group 4 Patients studied before and after radiation of the thyroid (4 cases)

Cases I and II were in definite negative calcium and phosphorus balance before and definite positive calcium and phosphorus balance after radiation Case III was in positive calcium and phosphorus balance before but in negative calcium and phosphorus balance after two courses of deep radiation This patient was subsequently operated on, but within six months of operation had another course of deep radiation because of a recurrence of the hyperthyroidism On the evidence of the clinical condition and the basal metabolism, the x-ray seemed to stimulate the whole thyroparathyroid apparatus in this patient

The fourth case was aged 14 She was in gross negative calcium and phosphorus balance before x-ray and was still in definite negative calcium and phosphorus balance after x-ray, but as only two months had elapsed since the radiation it was probably too early to get the final effect of the rays on the parathyroid glands

Nitrogen balance in the material studied

We endeavored to have each patient in nitrogen equilibrium but were not always successful, this is the most serious objection to using a single four-day period experiment If such a means of investigation should become a routine, larger nitrogen intakes are essential In our material, out of thirty-four periods there was positive nitrogen balance or equilibrium (less than —1 0 gram per day) in twenty cases and a loss of 2 grams or less a day in eight cases The greatest loss was in the 14-year-old patient, who lost 5 grams of nitrogen a day There can be little doubt that a definite negative nitrogen balance disturbs calcium metabolism and introduces complications into the interpretation of the results However, in the present study if we excluded from our data all the patients who had a negative nitrogen balance greater than 2 grams a day—Group 1, Case I, Group 2, Case II, Group 3, Case IX (two periods out of five), Group 4, Cases II and IV—the general tenor of the results would in no wise be affected

DISCUSSION

There is in general a negative calcium and phosphorus balance in untreated hyperthyroidism The negative calcium balance, however, is not invariable, and its extent bears no relationship to the amount of circulating thyroxin as measured by the level of the basal metabolism The administration of iodine, provided it does not bring the metabolism to normal, has no apparent influence on the state of the calcium and phosphorus balance Therapeutic radiation (deep or superficial) to the neck determines in the majority of cases a profound change in the calcium and phosphorus metabolism, two to three months after irradiation the calcium and phosphorus balance tends to become positive irrespective of the level of the basal metabolism at the time the balances are estimated In the series of patients presented in this paper the calcium and phosphorus balances were positive in twelve out of fifteen patients The experiments were conducted two or more months after irradiation and at the time the basal metabolism was definitely raised in each case

The most probable explanation of the negative calcium and phosphorus balance in untreated hyperthyroidism and the shift towards a positive balance in cases who have received radiation is that in hyperthyroidism the parathyroid glands undergo varying degrees of hyperplasia and that radiation has a more profound effect on hyperplastic parathyroid tissue than on hyperplastic thyroid tissue Another possible explanation is that the thyroid gland secretes a second hormone and that the cells responsible for its production are more radio-sensitive than the acinar cells that produce thyroxin It seems unnecessary to dis-

cuss the possibility of the pituitary gland being involved

Cope and Donaldson, in a recent article in this Journal (2), discussed the calcium and phosphorus metabolism of a patient who had a recurrent hyperthyroidism (postoperative) associated with hypoparathyroidism This patient was found to be in calcium and phosphorus equilibrium at a time when the basal metabolism, as a result of the administration of potassium iodide, was somewhat below normal, and in negative calcium and phosphorus balance when the basal metabolism rose after the potassium iodide was left off

The authors interpret their findings as follows "The studies of the calcium and phosphorus balance made on this patient confirm the findings of Aub and his coworkers The marked increase in calcium and phosphorus excretion to above normal, occurring during the time of increased metabolic rate with signs of thyrotoxicosis and continued tetany, lends substantial support to the belief that the increased excretion in hyperthyroidism is not due to a concomitant overactivity of the parathyroid glands"

There is an obvious increase in the calcium and phosphorus excretion in Cope and Donaldson's patient when the basal metabolism was raised, but there are possibilities to be considered other than that this increased excretion of calcium was due to a mobilization of calcium resulting from the "specific effect of the thyroxin circulating in excess" (3) Firstly, the diet being practically the same as that given when the basal metabolism was low led to an increased negative nitrogen balance, and a consequent large mobilization of phosphorus to which Cope and Donaldson refer, the increased acidity of the organism thereby determined would affect calcium metabolism The degree of tetany was not the same on the two occasions, during the experi-

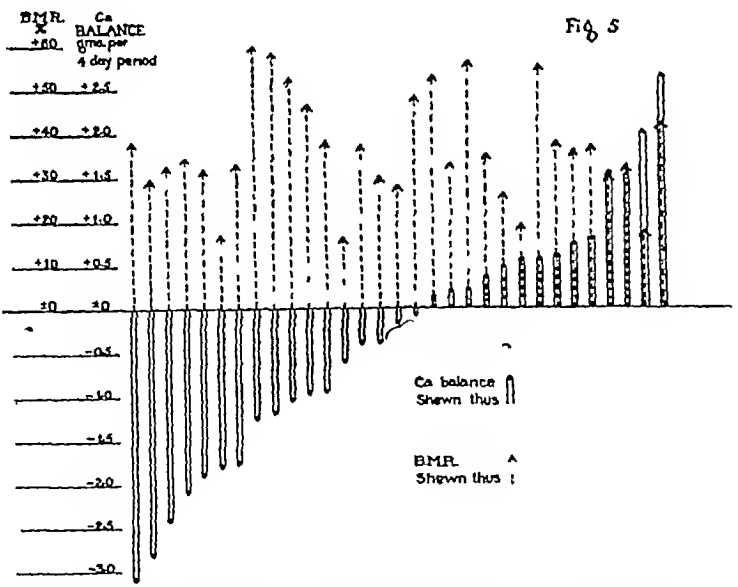


Fig. 5

FIG. 5 STATE OF CALCIUM BALANCE AND BASAL METABOLIC RATE (DURING THE EXPERIMENTAL PERIOD) ON THE SAME ORDINATE. 31 OBSERVATIONS ON 26 CASES GROUPS 1 TO 4 INCLUSIVE. THE CHILD AGED 14 CASE 4 GROUP 4 EXCLUDED

The cases are charted in descending order of calcium loss. This graph demonstrates the absence of correlation between the state of the calcium balance and the degree of hyperthyroidism as measured by the basal metabolic rate.

mental period when the basal metabolism was low there was active tetany, during the period when the basal metabolism was raised the tetany was practically absent

The results of the experiments on this patient are equivocal, they may denote a specific effect of the increased circulation of thyroxin on bone salt, as Cope and Donaldson interpret them, or the results may be simply an expression of the summation of three other factors, undernutrition, acidosis, and increased activity of the parathyroid glands, each of which of themselves are conducive to calcium loss

When the results obtained in the present study are considered, the same ambiguity does not arise Here we have a range of calcium balances from —3 1 grams of calcium per four-day period to +2 65 grams per four-day period, with an excess of circulating thyroxin in each of the thirty-five experimental periods The results of all the observations are charted in Figure 5 Statistically[8] there is no correlation between the level of the basal metabolism and the state of the calcium balance The evidence is complete that thyroxin *per se* has no effect on calcium catabolism The results do not prove that variation in parathyroid function is the true explanation of the observed phenomenon, but in the present state of our knowledge it is the most logical thesis

Physiological requirements of diets for use in calcium and phosphorus studies on human beings

It is important to consider the physiological requirements of diets for use in calcium and phosphorus studies on human beings In experimental work, standard conditions are essential, but in metabolic studies there must be variables, if a constant diet is used that is inadequate in various ways, the nutrition of different individuals suffers to a varying extent, and in turn the metabolism of substances under investigation may be disturbed If the components of the diet are kept constant, but change quantitatively though proportionally to meet caloric requirements, a variable is introduced, as the subjects are not examined on the same diet

The calcium content of the diet of the present study approximates that of "normal diets," and is greater than that employed by Bauer, Albright, and Aub (3) Its use demonstrates that in hyperthyroidism a wide range of calcium balance is possible The state of the calcium balance is independent of the degree of hyperthyroidism or the actual calcium content of the diet, *i e*, the state of calcium balance is apparently not directly influenced by the calcium content of the diet In some patients in whom the metabolism remained at a comparatively constant elevation, and the calcium content of the diet also remained constant, the calcium balance was observed to pass from gross negative to definite positive balance. When hyperthyroidism is associated with a negative calcium balance, there is an apparent inability to assimilate the calcium of the food quite apart from the mobilization of calcium from the bones, or in other words, there is calcium diarrhea.

A standardized procedure for use in various laboratories would be very valuable, as the results of experiments on calcium and phosphorus balance must vary when diets are used as divergent in principle as that of the Boston School and that of the present study

[8] See appendix for statistical analysis of this data.

SUMMARY AND CONCLUSIONS

1 Untreated hyperthyroidism is generally but not invariably associated with a negative calcium and phosphorus balance

2 There is *no* relationship between the level of the basal metabolism and the amount of calcium and phosphorus excretion

3 The oral administration of Lugol's iodine has no specific effect on calcium and phosphorus metabolism

4 Irradiation of the thyroid region in hyperthyroidism leads to profound changes in calcium and phosphorus metabolism In the majority of patients calcium and phosphorus equilibrium or a positive calcium and phosphorus balance occurs two months or more after the irradiation

5 The change in calcium and phosphorus balance that follows irradiation of the thyroid region is independent of the activity of the thyroxin-producing mechanism

6 The most likely explanation of the changes in calcium and phosphorus metabolism in hyperthyroidism and of the effect of irradiation is that in hyperthyroidism there is an associated hyperparathyroidism and that the hyperplastic parathyroid glands are radio-sensitive

TABLE I

Calcium and phosphorus balance of hyperthyroidic patients before treatment and on sodine therapy

Case number and patient's name	Age (years)	Sex	Date of experimental period	Before experimental period: Basal metabolic rate (per cent) Months 12	9	6	5	4	3	2	1	0	Experimental period: Calcium (grams per 4-day period) Intake	Urine	Stool	Balance	Phosphorus (grams per 4-day period) Intake	Urine	Stool	Balance	Nitrogen balance	After experimental period: Basal metabolic rate (per cent) Months 0	1	2	3	4	6	12	15	24	27	Remarks
GROUP I—BEFORE TREATMENT																																
I—Ho.	21	♀	Jan. 10–13, 1934								+22	+23	3.17	2.37	3.34	−2.44	4.23	4.47	1.92	−2.16	−12.3										Treated with iodine after experimental period, and then thyroidectomy. Histology—diffuse hyperplastic goiter
II—Bu.	31	♀	Feb. 3–7 1935				+39				+76	+60	2.73	0.93	3.64	−1.84	4.06	3.05	1.81	−0.80	+ 1.2								+41	+19 Iodine	Thyroidectomy June 6, 1937. Histology—patchy hyperplasia.
III—Mc.	36	♀	Dec. 6–8, 1934								+21	+16	3.93	1.25	3.28	−0.60	5.09	2.93	1.84	+0.32	+17.3					+31					Had deep x-ray but no further B.M.R. after the one shown.
IV—Bu.	36	♀	Jan. 26–29, 1935								+75	+48	3.66	0.99	2.91	−0.06	4.74	3.31	2.07	−0.56	+ 2.9										Patient lost sight of.
V—Do.			Aug. 19–22, 1935								+58		2.93	1.45	2.87	−1.19	3.87	2.52	1.52	−0.49	+ 2.0										Death on Sept. 27 1935. This patient had a very large goiter consisting of multiple adenomata. She had chronic bronchitis and severe myocarditis with auricular fibrillation. Autopsy: Thyroid gland weighed 590 grams, surface was very lumpy several of the lumps felt calcified. On section the thyroid gland was made up of very numerous adenomata which were of colloid type. They contained many large alveoli full of thin, sticky colloid as well as some small alveoli. Some were fibrous and calcified in the center. One series of adenomata in the upper pole of the right lobe contained some cysts full of thin brown fluid with cholesterol crystals. The thymus was enlarged. Histology of thyroid gland: Multiple adenomata, many of colloid type; some showed hyperplasia. Tissue removed as ? parathyroid glands turned out histologically to be lymph glands.

TABLE I—*Continued*

Case number and patient's name	Age (years)	Sex	Date of experimental period	Before experimental period: Basal metabolic rate (per cent) Months 12	9	6	5	4	3	2	1	0	Experimental period: Calcium (grams per 4-day period) Intake	Urine	Stool	Balance	Phosphorus (grams per 4-day period) Intake	Urine	Stool	Balance	Nitrogen balance	After experimental period: Basal metabolic rate (per cent) Months 0	1	2	3	4	6	12	15	24	27	Remarks
GROUP 2—ON IODINE THERAPY																															
I—McL.	28	♂	July 28–31, 1935						←		+32 Iodine	+38 →	3 48	2.78	3 91	−3.21	4.33	5 00	2.48	−3 10	− 1.5		+59	+64 Deep x-rays	+58	+67						This patient had iritis and a corneal ulcer He became extremely ill. B.M.R. rose to +67 per cent 4 months after experimental period. Deep x-ray to thyroid and later to pituitary region was tried. Ligation of superior thyroid arteries.
II—Da.	42	♀	Dec. 14–17, 1935							←	Iodine	+32 →	2 92	0.97	3.85	−1.90	4 00	3.50	2.37	−1.87	−12.3		+63	+47								Patient died of acute thyrotoxicosis with mental symptoms. No autopsy allowed.
III—Wa.	28	♀	Dec. 31, 1935–Jan. 3, 1936							Iodine	+74 Iodine	+33	3 56	1 77	3.58	−1 79	4.55	3 66	2 18	−1.29	− 6 7		+8	+30	+9							Operation when B.M.R. fell to +9 per cent. Histology—hyperplastic goiter, not quite diffuse—some fibrosis.
IV—Va.	22	♀	Jan. 18–21, 1935	←			Iodine				→	+38	3.54	0 90	3 48	−0 84	4.03	3.32	2.27	−0 96	− 0.2	←	Iodine	→							Deep x-ray therapy after experimental period.
V—Co	43	♀	Jan. 10–13, 1936								+20 ← Iodine	+17 →	3 17	1.26	3 72	−1.81	4.23	3.80	2.41	−2.04	− 7 6		+35	+25								Operation Jan. 21 1936. Histology—multiple adenomata with some hyperplasia.
VI—Du.	47	♀	Dec 31, 1935–Jan. 3, 1936							+37 ←	+17 Iodine	+30 →	3.56	1.01	2.59	−0 04	4.55	3.50	1 60	−0.55	+ 0.3	+40										Auricular fibrillation. Operation Jan. 21, 1935. Histology—multiple adenomata with some hyperplasia.

TABLE II

Calcium and phosphorus balances of hyperthyroidic patients after therapeutic irradiation of the thyroid region and before and after therapeutic irradiation of the thyroid region.

Case number and patient's name	Age (years)	Sex	Date of experimental period	Before experimental period: Basal metabolic rate (per cent) Deep x-ray (dose to each lobe) — Months: 36	30	24	21	18	15	12	9	6	5	4	3	2	1	0	Experimental period: Calcium (grams per 4-day period): Intake	Urine	Stool	Balance	Phosphorus (grams per 4-day period): Intake	Urine	Stool	Balance	Nitrogen balance	After experimental period: Basal metabolic rate (per cent) X-ray (dose to each lobe) — Months: 0	1	2	3	6	12	18	24	36	Remarks
GROUP 2—AFTER THERAPEUTIC IRRADIATION OF THE THYROID REGION																																				
I—Ca.	29	♀	July 7–10, 1935								+57 1400 r.	+44	+64 1400 r	+33	+89		+29	+15	4.09	0.38	1.61	+2.10	4.67	2.21	0.98	+1.38	+18.1										Developed symptoms of hypoparathyroidism, relieved by vitamin D. Went to England 2 months after experimental period.
																	+30	4.09	0.22	2.36	+1.51	4.67	3.21	1.58	−0.10	+ 6.0	+32					− 1		−10		Seen 2 years after experimental period. Thyroid palpable, not vascular. Patient very well. This patient had been too ill for operation before x-rays.
II—St.	20	♀	July 7–10, 1935					+65	+50 1400 r	+68	+35 2900 r	+57																									
																	+35	3.19	0.96	1.50	+0.73	4.21	2.49	0.94	−0.22	+ 1.6										This patient did well after 1st course of deep x-ray and then relapsed. Was referred from another hospital. B.M.R.'s done at Royal Prince Alfred Hospital.
III—O'C.	25	♀	Nov 24–27 1935						+36 1200 r	− 4	+60 1500 r.																									
															+25		+35	3.19	0.51	1.99	+0.59	4.21	3.49	0.97	−0.25	− 5.7										Clinically this patient was still hyperthyroidic 20 months after experimental period. We could not get co-operation for a B.M.R. owing to mental deterioration.
IV—Pe.	45	♀	Nov 24–27 1935		+80 1200 r	+66 1600 r			+10	1200 r	+33																								1200 r.	
												+46					+55	2.93	0.50	2.78	+0.57	5.14	2.70	1.41	+1.03	+12.4										Private patient, extremely "highly strung." Ultimately did very well.
V—Wl.	47	♀	Sept. 28–Oct. 1, 1935									1400 r.	1400 r.																				+59		+33 1600 r	+43	
																	+57	+25	3.52	0.99	2.18	+0.33	4.43	2.64	1.24	−0.40										Originally this patient had been extremely ill and deep x-ray was given as a "forlorn hope." Thyroidectomy Nov 5, 1935. Histologically moderate to pronounced hyperplasia (patchy).
VI—Fa.	29	♀	Feb. 14–19 1935								+78		1500 r		1400 r.																					
																	+56	4.18	0.40	3.57	+0.21	3.53	4.28	1.98	−0.79	− 0.4	+33	+23								
II—Sh.	28	♂	Oct. 12–15, 1935								+50 2000 r		+43			1500 r																					

STATISTICAL ANALYSIS

(By W A Carr Fraser)

Correlation between the state of the calcium balance and the basal metabolic rate of the material of the present study

The thirty-one experimental periods can be divided into two groups according to the state of the calcium balance (*1*) 14 periods showing positive calcium balance per four-day period (*2*) 17 periods showing negative calcium balance per four-day period The correlation between the state of the calcium balance and the basal metabolic rate of these two groups can be investigated Following Fisher's method (Section 34 (4)) significance can be attributed to a value of a correlation coefficient derived from a sample when a correlation coefficient as large as the one found would be obtained at most once in every twenty or more random samples of similar size from an infinite population which showed zero correlation

Subjects in positive calcium balance per four-day period

The correlation coefficient between the state of calcium balance and the basal metabolic rate is —0 33 A correlation coefficient as large as this would be obtained in one out of every four random samples of the 14 pairs of observations drawn from an infinite population showing zero correlation On the evidence, therefore, there is zero correlation between the basal metabolic rate and the state of the calcium balance per four-day period for subjects in positive calcium balance

Subjects in negative calcium balance per four-day period

The correlation coefficient between the state of calcium balance and the basal metabolic rate is + 0 18 A correlation coefficient as large as this would be obtained in approximately one out of every two random samples of 17 pairs of observations drawn from an infinite population showing zero correlation On the evidence, therefore, there is zero correlation between the basal metabolic rate and the state of the calcium balance per four-day period for subjects in negative calcium balance

Combination of the two groups

Having shown that each group is equivalent to a random sample drawn from an infinite population showing zero correlation between the state of the calcium balance per four-day period and the basal metabolic rate, we can estimate the weighted correlation coefficient of the two samples according to the method of Fisher (Section 35, example 33 (4)) This estimate gives —0 05 for the correlation between the state of calcium balance and the basal metabolic rate A correlation coefficient equal to this figure would be obtained in eight out of every ten random samples of this size by sampling an infinite population of which the correlation is zero

CONCLUSION

This investigation shows that there is no correlation between the state of the calcium balance per four-day period and the basal metabolic rate.

BIBLIOGRAPHY

1 Hansman, F S, and Wilson, F H, Calcium and phosphorus metabolism in diseases of the thyroparathyroid apparatus I Calcium, phosphorus and total metabolism in hyperthyroidism and the part played by the parathyroid glands M J Australia, 1934, 1, 37

2 Cope, O, and Donaldson, G A, Relation of thyroid and parathyroid glands to calcium and phosphorus metabolism. Study of a case with coexistent hypoparathyroidism and hyperthyroidism J Clin Invest., 1937, 3, 329

3 Bauer, W, Albright, F, and Aub, J C, Studies of calcium and phosphorus metabolism II The calcium excretion of normal individuals on a low calcium diet, also data on a case of pregnancy J Clin. Invest, 1929, 7, 75

4 Fisher, R A, Statistical Methods for Research Workers—Oliver and Boyd, London, 1936, 6th ed, revised and enlarged

STUDY OF THE TENDENCY TO EDEMA FORMATION ASSOCIATED WITH INCOMPETENCE OF THE VALVES OF THE COMMUNICATING VEINS OF THE LEG OXYGEN TENSION OF THE BLOOD CONTAINED IN VARICOSE VEINS

By H. E. HOLLING,[1] H. K. BEECHER, AND R. R. LINTON

(From the Surgical Laboratories of the Harvard Medical School at the Massachusetts General Hospital, Boston)

(Received for publication March 25, 1938)

The complications of varicose veins are well known, but the etiology of these complications has never been satisfactorily explained. Many more or less plausible statements as to the causes of varicose ulcer, for example, have been offered. One of the most persistent concepts has been that the incompetence of the valves of the leg veins is followed by venous stagnation, and venous stagnation by anoxia of the region, thus renders the tissues more susceptible to trauma and ulceration. The question remains, why do ulcers develop in one case but not in another? Evidently several factors are involved. This study was designed to add objective information regarding alterations of physiological processes which may be responsible for the complications associated with varicose veins.

deTakáts and his coworkers (1) and Blalock (2) have studied the oxygen tension of the blood found in varicose veins, to obtain data concerning the pathology associated with these abnormal veins. deTakáts found the oxygen content distinctly lower in varicose veins of the leg than in the antecubital veins of the arm. This is hardly a fair comparison. Blalock found "... in nine of the ten cases observed, the oxygen content of the blood from the femoral veins was higher on the diseased side ... than on the opposite side." He stated, "No definite relationship seems to hold between the oxygen content of the blood of the dilated veins of the lower part of the leg and similarly located normal veins of the opposite leg ..."

We questioned this latter statement and expected to find on examining the blood a greatly lowered oxygen content in the varicose veins. We must admit at once that no significant lowering of the oxygen content was found when analyses of blood from varicose veins with and without ulceration were compared with analyses of blood from similarly situated normal veins. When ulcers were present, blood was usually withdrawn from the vein draining the ulcer. It was taken under oil. Heparin was used as the anticoagulant. The blood was aspirated in as small quantity as was consistent with careful analysis (not more than 3 cc.). Small quantities are important to avoid the possibility of withdrawing blood contained in the deep veins, where presumably the oxygen content would be higher.

The oxygen content and the total oxygen combining power were determined in duplicate by the method of Van Slyke and Neil (3), and from these results, the oxygen tension of the blood was calculated from the data of Henderson, Bock, *et al.* (4). These data are presented in Tables II, III, and IV.

The failure to find evidence of hypo-oxygenation of the blood in varicose veins was unexpected, and it was thought that rest in bed might have had a temporarily beneficial effect on the venous circulation of the legs. This was disproved by taking blood from the varicose veins of ambulatory patients in the Outpatient Clinic and comparing the oxygen content with that of blood from normal leg veins under similar circumstances.

Superficially, it would seem that the major premise of the explanation outlined above regarding the cause of varicose ulcers had been destroyed by Blalock's observations supported by our work. Actually, it must be kept in mind that the oxygen content of the blood in the large veins may not give a true representation of the capillary oxygen content. One uncontrolled factor here is the possibility of a shunt of blood directly from the arterioles to venules, largely avoiding some regions of the capillary bed. Furthermore, pressure from edema of the skin would seriously impair the local capillary circulation there. Accordingly, we were

[1] Fellow of the British Medical Research Council.

led directly to a study of edema formation in cases of varicose veins with and without ulcer formation

Measurement of edema formation

In order to measure the amount of tissue fluid formed in the legs, the patient was taken from bed and seated for 2 hours with his foot in a dependent position The increase in volume of his leg, after the circulation had become stabilized, was taken as a measure of the amount of tissue fluid formed

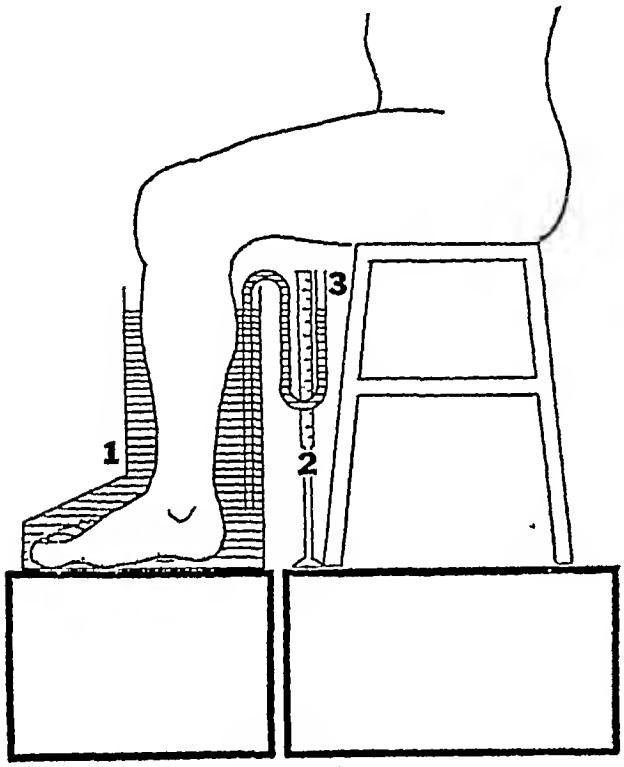


FIG 1

APPARATUS FOR MEASURING THE VOLUME OF TISSUE FLUID FORMED DURING TEST

To measure the leg volume a boot-shaped plethysmograph of 9 liters capacity was made from reinforced copper sheet. This was insulated with felt and plaster bandage When the leg was placed in the boot care was taken that the circulation should not be obstructed by clothing, by pressure of the sides of the plethysmograph, or by the chair edge With the foot in position, 5 liters of water at 86 to 90° F were poured into the boot. After allowing 10 minutes for the relaxation of the arterioles, veins, and smaller vessels (5) the height of the water in the plethysmograph was measured. The most satisfactory method was found to be by the use of a siphon and U-tube manometer arranged as shown in Figure 1 The volume of the leg was measured by water displacement. Then all of the water was siphoned off, so that the hydrostatic pressure of the water on the leg should not hinder the formation of edema during the observation period.

The plethysmograph was placed on a separate platform so that it could be lowered from the leg, emptied, and the leg dried gently without moving it This prevented emptying of the veins by leg movement.

At the end of 2 hours, 5 liters of water at the same temperature as that used at the beginning of the experiment were poured into the boot As a result of the transudation of fluid from the blood stream into the tissues, the leg volume was increased and the water rose to a height greater than the initial level Water was removed until the original level was regained. That removed was measured. Its volume represents the quantity of edema fluid which was formed during the sitting period, and is expressed as cubic centimeters of tissue fluid per 100 cc. leg volume It is important to have the foot and leg in exactly the same position for each of the two determinations

The accuracy of using the manometer as a measure of the height of the fluid in the boot was tested by adding measured amounts of water to that in the boot and then measuring the volume of water it was necessary to remove to bring the manometer reading back to the previous level The actual amount of water added was not known by the observer The results given in Table I show that the error was small enough to make significant the changes in volume found

TABLE I

Accuracy of measurement of changes in the water level in the boot

Actual volume of H_2O added	Volume of H_2O removed to readjust manometric level
cc	*cc*
20	19
11	11
50	53
15	14
20	20
30	29
25	27

RESULTS

The detailed results obtained in the study of edema formation in these cases are shown in the tables The amount of tissue fluid formed in 2 hours in the legs of 4 *normal* individuals was 3.9 ± 0.7 cc per 100 cc leg volume

Six patients having varicose veins without ulceration showed an edema formation in 2 hours of 6.0 ± 0.7 cc per 100 cc leg volume

Five patients having varicose veins with ulceration showed an average edema formation of 6.5 ± 0.6 cc per 100 cc leg volume

It is important to make a series of such studies at the same time of year if they are to be com-

TABLE II

Normal subjects

Patient	Sex	Age	Amount of edema per 100 cc. leg	Blood oxygen before and after sitting	Blood oxygen at saturation	Oxygen saturation	CO_2 volume	Oxygen tension	Comment (Patients sitting unless otherwise specified)
		years	*cc*	*cc per 100 cc*	*cc per 100 cc.*	*per cent*	*per cent*	*mm Hg*	
M M	M	50	5.5	8.5 12 4	19 0 22 8	45 0 54.5	58 8 53 5	25.3 29 6	No abnormality of circulation of the legs
B T	M	50	3.3	13 4 9.3	19.2 19 7	69 0 47 4	60 5 64 0	39 5 28 2	No abnormality of circulation of the legs
H E. H	M	28	2 2	15 9 16.2	18 1 19 9	87.5 81.3	51 0 50 7	54 5 47 0	No abnormality of circulation of the legs
H E. H	M	28	(4 4)*	13 8 16 2	19 9 22 0	69.5 73.5	56 0 46 0	38 4 39 0	No abnormality of circulation of the legs (staoding)
C. S	M	19	4 6	18 6 16 1	22 2 22 9	83 9 70 2	50.3 52.5	49 0 38 3	Tendency to acrocyanosis shown in feet circulation of leg other wise oormal

* Not rocluded in the mean because subject was standing
† The standard deviation of the mean for the edema fluid is 3 9±0 7

pared, for studies made in hot weather are not comparable to others made in cool weather

The above results show a barely significant increase in the formation of tissue fluid in the legs in which simple varicose veins are present, for the difference of the means is a little more than twice the square root of the sum of the squares of the standard deviations There is no significant increase in the formation of edema fluid in the patients having ulceration with their varicose veins over the varicose group not having ulceration.

The communicating veins are the vessels which connect the superficial and deep systems of the leg veins In the normal leg the valves of the communicating veins above the ankle are arranged so as to permit the flow of blood inward The incompetence of the valves of these veins can be demonstrated (Trendelenburg) if the leg is first elevated to empty the veins and a venous tourniquet then applied below the knee. When the leg is allowed to become dependent, the superficial veins fill rapidly from the deep veins if the valves of the communicating veins are incompetent If the valves in the communicating veins are competent the superficial veins fill slowly by the venous return from the foot but rapidly from the incompetent saphenous system as soon as the tourniquet is released.

All the cases of varicose ulcer which we examined in this series showed incompetence of the valves of the communicating veins Occasionally (Table III) incompetence of the valves of the communicating veins was found in the group without ulcer

When the subjects are divided into two groups on the basis of whether the valves of their communicating veins are competent (normals plus those with simple varicosities of the great saphenous system) and those in whom the valves of the communicating veins are incompetent, a highly significant increase in the formation of edema fluid is found in the latter group

The following subjects fall into the first group Subjects M M, B T, H E H, C S, M E W, M F, and W L The edema fluid formed per 100 cc. leg volume in 2 hours of sitting is 4 1 ± 0 4 cc.

These subjects fall into the latter group Subjects M M L, M C., C J B, M K., B M, E M, E C S, and B E F The edema fluid formed per 100 cc leg volume in 2 hours of sitting is 6.9 ± 0 4 cc.

The difference of the means here is 5 times the square root of the sum of the squares of the standard deviations of the mean, accordingly, the data are highly significant

We can say, therefore, that the rate of edema formation in cases of varicose veins appears to

THE DETERMINATION OF THE CARDIAC OUTPUT IN MAN AT BRIEF INTERVALS BY A MODIFICATION OF THE ETHYL IODIDE METHOD[1]

By J. C. SNYDER[2]

(From the Surgical Laboratories of the Harvard Medical School at the Massachusetts General Hospital, Boston)

(Received for publication April 1, 1938)

Ethyl iodide has been used extensively for the determination of cardiac output in man by the method of Starr and Gamble (1, 2, 3). In their procedure, the subject breathes from a spirometer for a total of 25 or 30 minutes during a determination in duplicate. Under many conditions a more rapid technique is required. For example, surgical patients may frequently be too sick in the first few days after operation to undergo repeated determinations involving a long period of breathing through mouthpiece and valves. Moreover, the rapidity of the circulatory changes during recovery from anesthesia demands a method of study which occupies less time than 15 to 20 minutes for a single estimation. This is particularly true of the changes induced by emotion or vigorous exercise.

Analysis of the air samples for a determination in duplicate by Cool's iodate technique (4) takes approximately 2 hours. The method is thereby restricted to very few determinations in any one day.

This report includes three sections. Section I describes a modification of Starr and Gamble's method which permits a determination in duplicate of cardiac output in 12 minutes, and additional determinations every 6 minutes thereafter. Section II presents a method of sampling which requires 45 minutes for the analyses involved in duplicate determinations, or 1 hour for triplicate determinations. Section III contains the data obtained on normal subjects and hospital patients using the modifications as described in Sections I and II.

I. MODIFICATION OF STARR AND GAMBLE'S METHOD

A. Alveolar air

(The reader is referred to Starr and Gamble's papers for a description of the principles and technical details of their method.)

In Starr and Gamble's procedure, the negative pressure during inspiration is utilized to withdraw the last few cubic centimeters of each expiration into a 500 cc. tube. A Bohr meter in the alveolar circuit is used to regulate the rate of collection of the sample. Approximately 2½ liters of air must be drawn through before constant composition is attained in the 500 cc. tube, the time required being about 12 to 15 minutes.

The principal change is a reduction of dead space in the alveolar circuit from 500 cc. to 15 cc. and a consequent reduction in the washing-out time from 15 minutes to 20 seconds. This is accomplished with the apparatus illustrated in Figure 1. A measured adjustable volume of mercury is allowed to flow out of a sampling tube during each inspiration, withdrawing the last portion of the previous expiration from the mouthpiece (1, Figure 4). Mercury is used instead of water to prevent loss of ethyl iodide and to facilitate rapid collection of the sample.

Manipulation of the apparatus in obtaining the alveolar sample. The person conducting the determination watches the water manometer continuously. A, B, and C are filled with mercury, up to K, before the determination is begun. With F midway between 1 and 2, M_2 is opened and the dead space is washed out by turning F to 1 during inspiration and to 2 during expiration. A convenient amount is 5 to 8 cc. per breath. Three such portions of air are withdrawn during 3 successive inspirations into C and discarded by ejection through the upper stopcock to room air. The sampling is immediately started by closing M_2 and opening M_1. When A is full, M_1 and D are closed. During the second determination, both C and B are taken, C for analysis of carbon dioxide as a check on the method of collection of alveolar air. When respiration is irregular, as it may be in anesthetized patients, great care must be used to avoid taking a portion of air immediately after a respiration that is appreciably more shallow than

[1] Aided by grants from the Josiah Macy Jr. Foundation and the William F. Milton Fund.

[2] Research Fellow in Surgery.

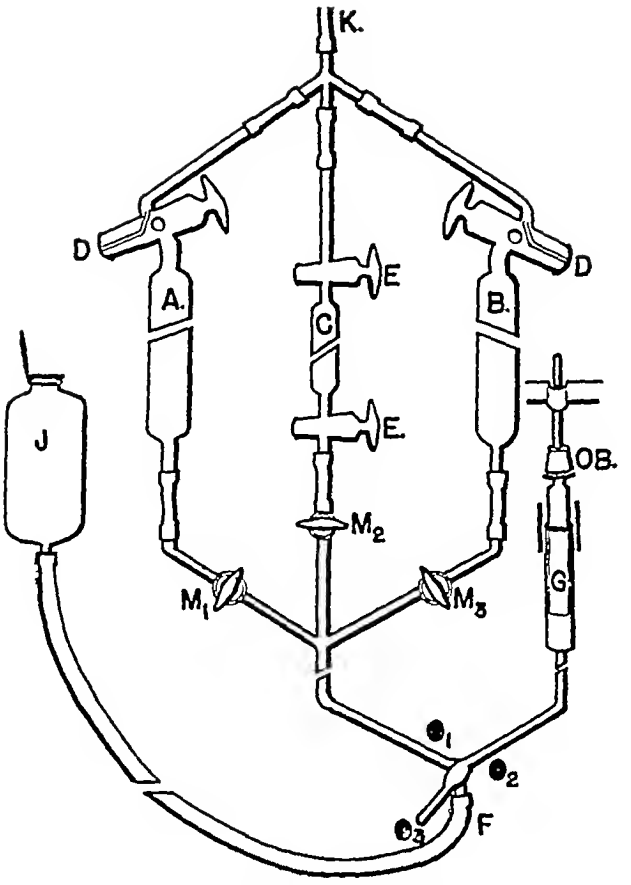


FIG 1 SCHEMA OF APPARATUS

A. and B are calibrated tubes containing 200 cc. and fitted with stopcocks, D D, which are inclined downwards about 30 degrees from the horizontal and are about 6 cm in length, with an outside diameter of 17 mm at the lower end The bore of the plug is 4 mm for the connection between the manifold K. and the tube. The hole in the center of the plug which connects either K. or the tube with outside air is 1 mm M_1, M_2, M_3 are 4 mm stopcocks All tubing is of 7 mm glass, with glass to glass connections, excepting that between F and J which is nitrometer rubber tubing C is a sampling tube of 60 cc capacity fitted with two three-way downward plug stopcocks, E. E., of 4 mm bore. F is a Y-stopcock, 4 mm bore, with a metal strip attached to the handle of the plug, in Position 1, the mercury is allowed to flow from any of the tubes A., B, or C into the Syringe G, in Position 2, the mercury flows from G into J, the dots at 1 and 2 indicate stops against which the metal strip on the stopcock plug handle strikes, thereby permitting the operator to control the flow of mercury without looking at the stopcock. Position 3 connects J and the tubes above during the transfer of samples G is a syringe barrel with an outlet of 7 mm., it is calibrated in 2 cc. amounts up to 20 cc. A collar of rubber tubing around the top permits a small amount of mercury to be added for a seal OB is an adjustable rubber obstacle against which the plunger of the syringe strikes when mercury is admitted from the tubes above. A water manometer connected directly to the inspired air line between the mouthpiece and the rubber valve is not shown. There is no Bohr meter or Mueller valve in the circuit.

the others, or after the subject swallows, since one obtains thereby dead-space air which is high in ethyl iodide content If the analysis for carbon dioxide in the alveolar sample is omitted, tube C may be used as a reservoir for dead-space air, a step which shortens the procedure by half a minute

With this apparatus it is not necessary to counterweight the spirometer which may be balanced so that the subject is entirely unaware of effort during inspiration

An important source of error in collecting the alveolar sample needs emphasis Condensation of water above the rubber valve in the mouthpiece may seal the opening of the alveolar line *at any* point in the collection of the sample A mercury manometer in the alveolar line immediately indicates occlusion of the orifice in the mouthpiece and permits adjustment of the valve at once

B Procedure

Starr and Gamble's procedure is followed in respect to the preliminary arrangements (1, 2) When the patient begins to breathe from the mouthpiece, the stopwatch is started and the spirometer reading is noted at the end of an expiration Spirometer readings are recorded at minute intervals Pulse rate and respirations are counted for 30 seconds alternately every other minute In the interval between 0 and 3 minutes, the rebreathing bag is charged with room air (Section I, C) Between 3 and 6 minutes the following operations are performed The I (inspired air) sample is taken and transferred (see Section II) The collection of the sample for determination of metabolic rate is adjusted so that the expired air from the center of the mixing bottle is drawn uniformly over the 3-minute period into the sampling tube The dead-space in the alveolar line is washed out and the A (alveolar air) sample is collected, but not transferred The E (expired air) sample is taken but not transferred As quickly as possible thereafter, the valve in the mouthpiece is turned to the rebreathing bag at the end of an expiration Time, spirometer reading,

and temperature are noted After the rebreathing is completed, the patient is disconnected from the apparatus or the second determination is begun without pause Regardless of which is done, the R (rebreathed air) sample is quickly taken and the E and R samples are transferred If the patient has started to breathe for the second determination these operations and any other maneuvres necessary to begin the subsequent test can be performed in the preliminary 3 minute period, which also allows time for complete recovery of the respiration and circulation from any changes caused by the short rebreathing period When the second estimation has been completed, both A samples are adjusted to the calibration mark at atmospheric pressure and transferred

A sample for determination of metabolic rate is collected during each test, the second being used for the analysis, the first only if the second fails to check satisfactorily

A mercury manometer was placed in the alveolar line to detect false alveolar samples The determination of carbon dioxide was omitted To determine the time required to wash out the dead-space in the expired line, room air was drawn through the apparatus for an hour, and a person of average size, under basal conditions, with a respiratory minute volume of 4 liters per minute, began to breathe through the mouthpiece. The per cent of oxygen in samples taken quickly from the center of the mixing bottle every minute showed no significant change after 3 minutes That interval was chosen for the routine preliminary period.

The spirometer readings at the beginning of the sampling and at the start of the R period are utilized for the calculation of the respiratory minute volume. If the readings at minute intervals indicate marked fluctuations, the determination is discarded

C The rebreathing period

Starr and Gamble (1, Figure 2) investigated the concentration of ethyl iodide in the rebreathing period after the subject had inspired from the spirometer mixture for 15 minutes They found a slow decline, the 30 second value being about 2 per cent less than the extrapolated 15 second value. When the period of breathing ethyl iodide is shortened to 6 minutes, it is possible that the tension of ethyl iodide in the venous blood will fall more rapidly during the R period than it does when the tissues are more nearly saturated with ethyl iodide. To obtain data on this point, the following apparatus was used. A manifold with capillary jets was inserted between the mouthpiece and R bag Mercury sampling tubes calibrated at a mark on the stem were evacuated and attached to the manifold. Samples of air from the lung-bag system were taken rapidly at suitable intervals in the R period, and were adjusted to the calibration mark (at atmospheric pressure) before transfer to titration bottles Results of 28 experiments on 2 normal subjects under basal and non basal conditions may be stated briefly When the bag contains a liter of air at the expected concentration of ethyl iodide and when the subject does not alter his respiration (rate 8 to 16 per minute) in any way in the R period, the concentration of ethyl iodide rises sharply to a peak, falls somewhat less rapidly to 15 or 20 seconds, then declines at a uniform rate to 30 or 35 seconds, and decreases more rapidly thereafter Under these circumstances, mixing probably does not occur before 20 seconds and samples up to that time do not indicate the vapor tension of ethyl iodide in the venous blood Between 20 and 35 seconds the slope of the curve varies considerably, sometimes there is almost no change, sometimes there is a fall at 30 seconds to a value as much as 10 per cent below the 20-second value. From these observations, it is concluded that the R period in Starr and Gamble's procedure must be modified to eliminate the possible variations when a 6-minute breathing period is employed instead of the 15 A more accurate R value can be obtained after a 6-minute period if the subject increases the rate and depth of respiration and terminates the rebreathing within 15 or 18 seconds, that is, before recirculation has occurred to an appreciable degree It was found convenient to have the subject empty the bag completely 5 or 6 times in response to spoken directions If room air is placed in the bag instead of a prepared ethyl iodide mixture, the time required for mixing is decreased

If the subject has been inhaling from the spirometer for twelve minutes, the ethyl iodide concentration in the R bag behaves in the same manner as described by Starr and Gamble (1,

Figure 2) When the subject is not disconnected from the spirometer between determinations, the above modification is not necessary in the second and third R periods, which may be conducted as in Starr and Gamble's procedure On numerous occasions observations in triplicate revealed no greater difference in the cardiac output between the first and third estimations than is expected from the ordinary variations of the method (see Section III)

II MODIFICATION OF COOL'S METHOD FOR THE DETERMINATION OF ETHYL IODIDE

The shaking and washing procedures in Cool's method for the determination of ethyl iodide (4) are eliminated as follows The sample is collected by water displacement in a stopcock tube calibrated at 400 cc (Figure 2) The source of air to be sampled is attached to arm A, the dead-space is drawn out through B, and the sample is admitted to the tube through arm C

The flat-bottomed titration bottle is illustrated in Figure 2 The bottle is prepared by adding 10 cc bromine water and evacuating with a water pump until the bromine water just begins to boil (Twice the quantity of bromine water recommended by Cool is used since some bromine vapor is removed during evacuation) A 4-inch section of rubber tubing (3 mm wall, 12 mm inside diameter) and a large hemostat or screw clamp seal the bottle [3]

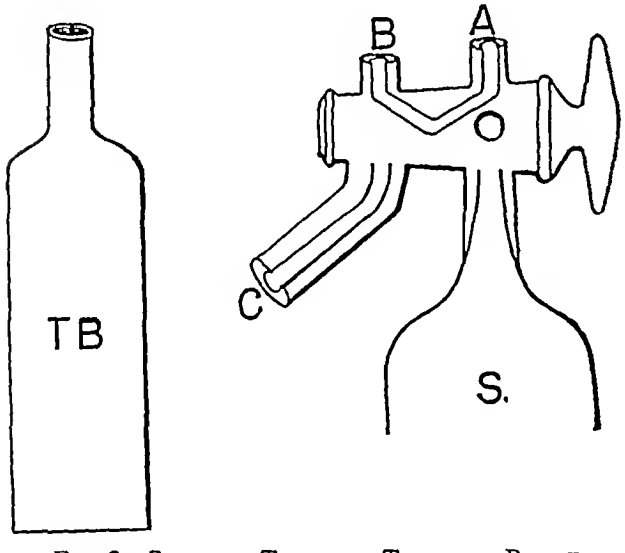


FIG 2 STOPCOCK TUBE AND TITRATION BOTTLE

S is a sampling tube calibrated at 400 cc. The stopcock is constructed to connect the tube, S, and the arm marked A , the tube and arm C , and arms A and B Arm C. is 17 mm in outside diameter, and has a bore of 2 mm. The stopcock bore between A and S is 4 mm., between A. and B (or C and S), 2 mm

T B is a flat-bottomed titration bottle which holds approximately 500 cc. The orifice at the top is 17 to 18 mm. in diameter, a size which conveniently permits introduction of reagents required by the titration procedure.

To transfer the sample from the stopcock tube to the titration bottle, the latter is fitted to arm C, the hemostat is removed, and the bottle is pushed up into contact with the arm of the stopcock, which is then turned to connect the tube and titration bottle Flow of water is controlled by a pinch clamp on the rubber tubing from the reservoir Care must be taken to prevent any water from entering the titration bottle, since varying amounts of ethyl iodide are introduced, depending upon the number of times samples have been admitted to the tube The rubber tubing is again securely clamped before the titration bottle is detached from *C* The remaining procedures are carried out in another room free of ethyl iodide vapor (or after the windows have been opened) in order to prevent traces of ethyl iodide from being drawn into the bottles when the tubing is removed for titration (the pressure inside the bottles will be somewhat less than atmospheric if leaks have been avoided) After rotation for a minute or two, the walls of the bottle are washed down with 20 cc distilled water, the remaining steps are carried out directly in the titration bottle following the directions given by Cool

The principal source of error in this method is the possibility of loss of ethyl iodide into the displacing fluid Starr and Gamble recommended (5, p 526) 0 5 per cent nitric acid for a displac-

[3] If the titration bottles are evacuated before the patient is allowed to breathe from the spirometer, it is advisable to seal the bottles with the blunt end of a test tube which is pushed part way into the rubber tubing before the hemostat is removed, and then into direct contact with the titration bottle. The 8 bottles necessary for duplicate determinations of output can be prepared easily in another room during the preliminary rest period while the patient is becoming basal If test tube plugs are not used, an occasional leaky screw clamp or hemostat may allow sufficient air to enter the titration bottle during the few minutes of the test to prevent the accommodation of the entire 400 cc. sample

ing fluid This was tried and found satisfactory at first After a considerable number of determinations had been run using the same acid, consecutive samples from the spirometer began to show wide fluctuations in ethyl iodide content. Satisfactory results were obtained by replacing the nitric acid with distilled water When freshly distilled water is the displacing fluid, analysis of air samples from the spirometer (containing ethyl iodide in the concentrations used in cardiac output determinations) regularly shows an average deviation of 0.9 to 1 5 per cent

Another inaccuracy may be introduced if the samples are held in the collecting tube for varying lengths of time before transfer to a titration bottle. When a determination of cardiac output is run in duplicate, all the samples collected over water are transferred within a minute, and each is exposed for the same time as the corresponding sample in the other determination The alveolar samples, being over mercury, are not transferred until a pair of tests is completed On three occasions a sample was left in the collecting tube for 5 minutes before it was transferred The values obtained did not differ from a previous series of samples by amounts in excess of the expected variation

Volume of samples The samples collected over water (I, E, R) are 100 cc smaller than those in Starr and Gamble's procedure The decrease in accuracy was accepted in the interest of convenience and rapidity The alveolar samples, over mercury were reduced to 200 cc. so that the amount of air taken with each respiration could be decreased A greater margin of safety in turning the Y stopcock is attained when only 5 to 10 cc. of air are collected per respiration instead of 10 to 20 cc. The samples over mercury can be adjusted to a known volume more accurately than those over water and show less variation

III CARDIAC OUTPUT STUDIES ON NORMAL SUBJECTS AND HOSPITAL PATIENTS

Starr and Gamble's procedure (1, 2) in preparing subjects for the determinations was followed On several occasions the subjects slept in the laboratory bed and were studied soon after awakening Hospital patients in most instances were familiarized with the apparatus by a dummy test conducted before the preliminary rest period, or under non basal conditions on a different day

Tables I and II contain data on the cardiac output of 58 duplicate determinations on 9 normal subjects and 17 hospital patients For the surgical patients, pre- and postoperative data are included for the purpose of enlarging the number of duplicate determinations used in calculating the average deviation of duplicate estimations from their mean No data on patients under ether anesthesia are included

TABLE I

Basal cardiac outputs on normal subjects

Subject number	Date (In 1937) and cardiac output (liters per minute)
1	Jan 14 4 1, 3 4 Feb 18 2 9, 2 7 Mar 25 2 7 3.3 May 7, 2 9 3 1 May 12 4 1, 3 6, May 26 3 7, 2 6
2	Dec. 8, '36, 4 6 4 2 Mar 4, 4.3, 4 2 Mar 19, 4.2 June 17, 4.3 3 9
3	Mar 1, 2 7 3 0, May 4 3 8, 3 4 May 7, 3 7 3 1
4	Feb 12, 3 6 3 6 Mar 30 3 7 3 5
5	Mar 27 3 8 4 2 4 6 Apr 17 4 1 4 4 4 6
6	Apr 27 3 8 4 1 May 21 4 6 4 8
7	May 28 4 1 4 2
8	May 13 5.3, 4 9
9	May 26 3 4 3 2

TABLE II

Basal cardiac outputs on hospital patients

Patient number	Date (In 1937) and cardiac output (liters per minute)
1	Jan 18 4 9 5 1
2	Feb 19 4 0 4.3
3	Jan 9 4 8 5 4 Jan 11 6.3 3 2
4	Jan 19 7 0 5.2 Jan 22 3 4, 3.5
5	Jan 29, 2 8 3 4 Jan 28 3.3 3 6 Feb 3, 2 8, 2 7
6	Mar 12 3 1 4 2
7	Mar 23 2 4 3 1 Apr 8 2.5 2 9
8	Apr 10, 3 4, 3 6 Apr 11 2 6, 3.3 Apr 13, 4 2, 3 1 Apr 23, 4 8 3 0
9	Apr 7 5 6 6 0
10	Apr 29, 3 3, 3 1 Apr 30 2 9, 2 7, May 6 3.3 4.3
11	Mar 24 3 6 3 9
12	Apr 29 3 1 3 0 May 29 4 2 4 4
13	May 18 3 2 3.5 May 20 3 4 3 5 May 24 3 4, 3.3 May 28 5 0 2 6
14	Apr 24 3 8 3 8 Apr 28 3 4 3 6
15	May 14 2.3 2.5 May 15, 2.5 2 2 3 5 May 17, 2 8 2 8
16	June 8 4 7, 4 8 June 9 5 8 4 4
17	June 25, 3 7 5 4, 2.5 June 26 3 9 4 0

For normal subjects the per cent average deviation of duplicates from their mean is 5 1 (19 duplicate and 2 triplicate estimations) The per cent deviation of each day s average about the mean of the daily averages is 5 4 (for the 6 normal subjects studied on more than one occasion)

For hospital patients the per cent average deviation of duplicates from their mean is 8 3 (34 duplicate and 2 triplicate determinations) On three occasions, widely divergent results were obtained, and it is believed that the discrepancies were due to excitement of the subjects, since the second of the pair was much lower in each case When these three pairs are omitted, the per cent average deviation of duplicates from their mean becomes 6 7 The per cent deviation of daily averages about their mean is 6 6 for 9 hospital patients studied on more than one occasion (24 determinations in duplicate, postoperative data obtained within 60 hours of operation and etherization are not employed in the calculations)

These results show that the present method as applied in the cases of this study is less accurate than Starr and Gamble's procedure For comparison, their figures are stated (2) average deviation of duplicates about mean, normal subjects, 3 45 per cent, average deviation of duplicates about mean, hospital patients, 6 45 per cent, average deviation of daily averages about their mean, normal subjects, 6 3 per cent

The differences may be attributable to the small number in this series, and the fact that most of the patients were awaiting a surgical operation, as well as the variations in procedure which are introduced

DISCUSSION

The changes in Starr and Gamble's method described in this communication are mechanical They are such that the method may be applied to a wider variety of conditions For example, five consecutive estimations of cardiac output can be made in 30 minutes, each one representing the circulation during a 3-minute period The labor and time required for the analysis of samples by the chemical method are reduced

The method involves more effort on the part of the persons conducting the test The possibilities of error in collecting the alveolar sample are more numerous and require more careful attention The rebreathing period in the first of a pair of estimations has been altered

It should be pointed out that any of the modifications here suggested can be adopted alone If it is held that the modified R period is undesirable, the objection can be entirely eliminated by waiting 15 minutes before taking samples for the first determination, additional determinations can still be made at 6-minute intervals as long as desired, the supply of glassware and the energy of the persons conducting the test being the only limiting conditions If the mercury-syringe-stopcock for collection of alveolar air is not employed, the use of titration bottles saves time and labor in analysis nevertheless And finally, if greater accuracy is required, larger samples and mercury as displacing fluid may be adopted at the expense of prolonging the estimations to 8 or 10 minutes each Unfortunately, the katharometer method for the analysis of ethyl iodide (6) can be applied to repeated estimations at short intervals only if sets of collecting tubes equal in number to the consecutive determinations desired are provided

As described, the method is particularly applicable to the study of cardiac output and oxygen consumption when these functions are undergoing changes which persist as long as 3 or 4 minutes

SUMMARY

A modification of Starr and Gamble's ethyl iodide method for the study of cardiac output in man is described (Section I) A shorter, less laborious method for the collection and analysis of air samples is presented (Section II) Studies on normal subjects and hospital patients are cited (Section III)

The changes permit a duplicate determination of cardiac output to be made in 12 minutes and additional estimations every 6 minutes thereafter

The method is somewhat less accurate than Starr and Gamble's procedure Its advantages and limitations are discussed

---

It is a pleasure to acknowledge the helpful suggestions given by Dr Isaac Starr, Dr C J Gamble, and Dr M D Altschule, and the technical assistance of Miss Virginia Dewey and Miss A B Mangiaracine

BIBLIOGRAPHY

1 Starr, I, Jr, and Gamble, C J, An improved method for the determination of cardiac output in man by means of ethyl iodide Am J Physiol, 1928, 87, 450

2. Starr Isaac, Jr, Donal J S., Margolies, A., Shaw, R., Collins, L. H, and Gamble, C. J., Studies of the heart and circulation in disease estimations of basal cardiac output, metabolism, heart size, and blood pressure in 235 subjects. J Clin. Invest., 1934, 13, 561

3 Starr I, Gamble, C J., Margolies, A., Donal, J S., Jr., Joseph N and Eagle, E. A clinical study of the action of 10 commonly used drugs on cardiac output, work, and size, on respiration, on metabolic rate, and on the electrocardiogram. J Clin. Invest., 1937, 16, 799

4 Cool R. D., Determination of small amounts of ethyl iodide. J Biol. Chem., 1932, 97, 47

5 Starr I., Jr., and Gamble, C. J., A method for the determination of minute amounts of ethyl iodide in air, water, and blood by means of its reaction with silver nitrate, and experiments bearing on the determination of blood flow by means of ethyl iodide. J Biol Chem., 1927, 71, 509

6 Donal, J S, Jr., Gamble, C. J., and Shaw R., The cardiac output in man. An adaptation of the katharometer for the rapid determination of ethyl iodide in estimations of cardiac output by the ethyl iodide method. A study of the effect of posture upon cardiac output and other circulatory and respiratory measurements. Am. J Physiol., 1934 109, 666

THE CARDIAC OUTPUT AND OXYGEN CONSUMPTION OF NINE SURGICAL PATIENTS BEFORE AND AFTER OPERATION [1]

By J C. SNYDER [2]

(From the Surgical Laboratories of the Harvard Medical School at the Massachusetts General Hospital Boston)

(Received for publication April 1, 1938)

The effects of anesthesia and operation on the circulation and metabolic rate of man have not been accurately determined. A few studies of cardiac output and oxygen consumption have been reported in relation to surgical procedures but the evidence is incomplete and inconclusive. Polano (1), using Broemser's method (2), has interpreted his data as indicating an increase in cardiac output in the immediate postoperative period Rehn (3), with the same technic, has described a decrease in cardiac output postoperatively in those patients who show signs of shock and collapse Broemser's method has been criticized on theoretical grounds (4) *Altschule and Volk (5), using* the ethyl iodide method, have described the changes in cardiac output and oxygen consumption which accompany total thyroidectomy, but they present no data for the immediate postoperative period Blalock and his coworkers have found that trauma and hemorrhage (acute experiments) depress the cardiac output of dogs (6), that etherization produces an increase in cardiac output and a slight decrease in oxygen consumption, and that under very deep anesthesia the cardiac output returns to normal or is decreased (7)

To determine more definitely the cardiac output and oxygen consumption of human subjects in relation to general surgical procedures, studies have been undertaken on hospital patients before operation, during recovery from anesthesia, and at suitable intervals thereafter

TECHNICAL DETAILS

Cardiac output The ethyl iodide method (8) as modified to permit rapid determinations in unconscious patients was used (9) Before this adaptation could be applied to the study of patients anesthetized with ether, it was necessary to determine whether ether vapor affects the accuracy of the chemical determination of ethyl iodide vapor in air samples, or the solubility of ethyl iodide in blood

A Ethyl iodide analysis in presence of ether vapor

The effect of ether vapor on the analysis of ethyl iodide by Cool s method (10) was determined by the following experiments

A solution of ethyl iodide was prepared by breaking an ampule containing a known weight of redistilled ethyl iodide under the surface of the solute in a partly filled, calibrated liter flask. Fifty per cent ethyl alcohol was found to dissolve the ethyl iodide more rapidly than water and did not affect the results This solution was pipetted directly into bromine water to compare the accuracy of this method for determining ethyl iodide with Cool's data. Differences between amounts of ethyl iodide expected and amounts obtained were 0.7, 0 1, and 0 1 per cent of the amount present Double-ended glass sampling tubes, 500 cc. capacity, were charged with ether vapor, 6 to 8 volumes per cent. The solution of ethyl iodide was pipetted into these tubes which were subjected to Cool's procedure. The discrepancy between amounts expected and obtained was 0 6 and 0 4 per cent in two experiments These differences are of the same order of magnitude as those described by Cool Additional experiments conducted with titration bottles (see (9), Section II) and known concentrations of ethyl iodide vapor in the presence of ether gave similar results Ether vapor was therefore considered to have no appreciable effect on the analysis of small amounts of ethyl iodide by the iodate method (The presence of ether vapor causes a return of the blue color a few minutes after a titration has been completed, so that uniformity of procedure in titrating samples is essential after potassium iodide has been added)

[1] Aided by grants from the Josiah Macy Jr Foundation and the William F Milton Fund.

[2] Research Fellow in Surgery

B Distribution coefficient

There is no reason to expect ether to change the solubility of ethyl iodide in blood Nevertheless, it was considered advisable to determine the distribution coefficient for the blood of etherized patients since the physiological adjustments which accompany anesthesia and hemorrhage might have a significant effect Preliminary experiments suggested that it was impossible to estimate distribution coefficients on etherized, preserved blood Pressure of work prevented making this estimation immediately in the postoperative period on the patients subjected to the other studies Therefore, blood samples from etherized patients not in the study group were analyzed in duplicate as soon as possible after being taken The following method was used

A small room kept at 36 to 38° C was equipped with a Van Slyke apparatus, the chamber of which was used as a tonometer Five cubic centimeters of blood were admitted to the dry chamber followed by 45 cc of gas prepared by adding ethyl iodide to the patient's expired air collected in an anesthesia bag at the time of the venipuncture The chamber was shaken vigorously for 3 or 4 minutes Its contents were brought to atmospheric pressure by opening the top stopcock briefly The chamber was shaken again for 15 minutes A 50 cc syringe was attached to the side arm of the Van Slyke chamber and the air was slowly admitted to the syringe with as little change in pressure as possible Another 50 cc syringe was attached and received 6 portions of room air shaken under reduced pressure with the blood in the chamber Reagents were added to the two syringes and the contents washed into Erlenmeyer flasks for the remaining procedures of the chemical analysis

Results comparable to Cool, Gamble, and Starr's data (11) were obtained on normal blood treated as above in duplicate, but without ether, 6 99, 6 92, 6 94, 6 42, for a red count of 5 5 million When ether vapor was present, however, the values of a duplicate estimation were more divergent The average of 6 experiments in duplicate with etherized blood analyzed within an hour of withdrawal was 6 37 for an average red count of 4 6 million, a value which agrees well with the average, 6 44, for a similar red count, obtained on 15 normal bloods by Cool, Gamble, and Starr The deviation of duplicates from their mean, 0 91, and the standard deviation about the regression line, 0 74, are higher than the corresponding figures, 0 36, and 0 41, in Cool, Gamble, and Starr's series However, it seems probable that etherization does not alter the distribution coefficient except by changing the red count The data on cardiac output obtained on etherized patients are therefore calculated on the basis of the distribution coefficient as established by the red count or the oxygen capacity of the patient's blood at the time of the determinations

Blood gases The usual manometric Van Slyke-Neill technic was employed for routine blood gas analysis (12) For etherized blood, Shaw and Downing's method was used (13) (In some instances the Hempel pipette was replaced with a capillary U-tube blown to the side arm of the Van Slyke chamber) Blood was collected in an oiled syringe, stored over mercury with dry heparin as anticoagulent, and analyzed in duplicate except as noted in the Appendix to Table I at the end of the paper

Arterial oxygen saturation If the arterial oxygen saturation of a patient is reduced during the period of recovery from anesthesia, the ethyl iodide technic cannot be applied for the study of cardiac output unless it is shown that equilibrium as regards ethyl iodide exists between simultaneous samples of alveolar air and arterial blood Data secured during recovery from anesthesia on the patient (Case 2) whose arterial oxygen saturation was below 91 per cent are indicated in the table but are not employed in the comparison of results of cardiac output Data for two patients are included although no estimations of arterial oxygen saturation were done, since normal values were obtained for every patient on whose blood duplicate determinations were available

Oxygen consumption was determined by the analysis of expired air drawn from a mixing bottle (14, p 562) , for samples containing ether vapor, a sulphuric acid absorber was used (15)

Blood loss at operation was estimated by a modification of Gatch and Little's method (16)

CLINICAL DETAILS

The patients were selected from the general surgical wards They had no cardiovascular dis-

ease and, with one exception (Case 2), presented no abnormality other than the local lesion There were 4 patients operated upon for carcinoma of the breast, 1 for tumor of the pancreas, 2 patients had perineorrhaphy, suspension of the uterus and appendectomy, 1 had only perineorrhaphy, 2 had gastroenterostomy Of these patients, one was anemic and required a preoperative transfusion (Case 2), two were considered to be emotionally unstable to a degree distinctly exceeding the normal (Cases 7 and 8), and one patient (Case 6), not studied preoperatively, went into shock during operation for which he received two transfusions Postoperative studies were made before and after a third transfusion, although at the time of the determinations the signs of shock were absent.

Studies of cardiac output and oxygen consumption under basal conditions were made on one or more occasions before operation Routine preoperative medication was employed, the dosage and time of administration are described in the Appendix to Table I All patients had gas-oxygen-ether anesthesia except Case 5 who was operated upon under local anesthesia only Postoperative studies were made as soon as possible after the dressing had been applied to the wound. The etherized patients were unconscious at the time of the determinations which were conducted with one or two variations from the procedures described in (9) An assistant was delegated to keep the lips of the patient firmly shut around the rubber mouthpiece throughout the tests In some instances a metal airway passing back of the patient s tongue was fitted directly to the mouthpiece The R period (see (9), Section I–C) was altered Instead of a liter of air, the R bag contained only slightly more than the tidal volume of the patient, so that the bag would be emptied with each breath The R period was terminated by having an assistant abruptly exert firm pressure on the sides of the patient s thorax during an expiration in order to push as much air as possible into the bag Subsequent studies were undertaken the morning following operation, again in 3 and 4 days and at discharge, unless complications intervened Postoperative medication in relation to the studies is cited in the Appendix to Table I An oral temperature of 101° F or higher, local wound infection, or evidence of infection elsewhere, served in all instances to interrupt or to terminate the observations Intravenous fluids, as required by routine postoperative care, were administered after the studies of cardiac output and oxygen consumption of the period of recovery from anesthesia had been completed Three patients (Cases 8, 9, and 10) were given constant intravenous fluids for several days postoperatively for the purpose of another study In no instances were estimations performed when they might conflict with the patient's well being

Blood gases were determined at the times noted in the table and appendix. Blood pressure was taken either during or immediately after the determinations of cardiac output The capillary red cell count or the venous oxygen capacity of each patient was determined once preoperatively and on the occasion of most estimations of postoperative cardiac output for use in obtaining the distribution coefficient from the data of Cool, Gamble, and Starr (11) Plasma volume studies were conducted on several of the cases immediately before and after operation by Dr J D Stewart His data appear in another communication (17)

RESULTS

The diagnoses, and the results of determinations of cardiac output, oxygen consumption, blood pressure, pulse, respirations, temperature, body weight, and venous oxygen capacity (or red count), are presented in Table I Other pertinent clinical data together with the time and duration of operation, the medication and fluids administered, are contained in the Appendix at the end of the paper

Figure 1 is a graph showing per cent change in cardiac output plotted against time for the nine patients who had ether anesthesia The average of the preoperative data for each patient is used to compute the per cent changes, with two exceptions (*a*) One patient (Case 8) who had duplicate determinations, on the morning of operation, was apprehensive and the average of the determinations was unduly high This value was not employed to calculate per cent change. Instead, the basal level which the cardiac output reached in the determinations most remote from the operation was employed (The cardiac output regularly returned to normal within a few days post

TABLE I

Original data and diagnosis of cases

Case number	Date	Condition	Hour	Cardiac output†	Pulse rate	Respirations	Blood pressure	Oxygen consumed†	Body weight	Body temperature‡	Venous oxygen capacity§	Diagnosis Type of operation Remarks
	1937			*liters per minute*	*per minute*	*per minute*	*mm. Hg*	*cc per minute*	*kgm*	*° F*	*volumes per cent*	
1	January 9	Basal	9 15 a.m	5 1	56	14	150–80	191	70 7	97 6		Carcinoma, left breast Radial mastectomy
	January 11	Basal	9 50 a m.	4 8	60	14		172		98.2		
	January 12	Preoperative basal	9 40 a.m.	*5.0*	64	14	130–80	185		97 6	*4.5*	
	January 12	Postoperative anesthetized	3 50 p m.		76	17	120–80	165		97 0 r	*3.9*	
	January 13	Postoperative 1st day	9 25 a.m	*2 0*	76	16		196		99 0 r	*4.0*	
	January 22	Postoperative 10th day 8 hours postprandial	2 50 p.m.	*5.3*	64	12		199		97.8		
2	January 19	Basal	9 00 a.m.	6 1	92	11	130–70	128		98 0	*3.2*	Carcinoma of stomach Subtotal gastric resection Posterior Polya anastomosis Death Jan. 23, bilateral bronchopneumonia
	January 21	Postoperative anesthetized	4 30 p.m.	5 5	124	19	90–50	225	66 9	100 0 r	*3.5*	
	January 22	Postoperative 1st day	9 45 a.m.	3.5	112	16	100–70	218		100 4 r	*3 1*	
3	January 28	Basal	9 15 a.m.	3.5	80	16	140–70	222		98 0	4.7	Carcinoma of breast. Radial mastectomy
	January 29	Preoperative basal	9.25 a.m.	3 1	94	16	136–80	201	61.5	97 4		
	January 29	Postoperative anesthetized	3 45 p.m.	2 1	130	20	100–62	180		100 4 r	*4.9*	
	January 30	Postoperative 1st day	9 30 a.m.	*1.9*	128	16	100–60	223		100 0	4 0	
	February 3	Postoperative 4th day	8 50 a.m.	2.8	88	14		229	57 6	97.8	*3.6*	
	February 9	Postoperative 11th day	9 20 a.m.	*3.2*	80	15		187	58 9	97.8		
4.	March 12	Preoperative basal	8 00 a.m.	3 7	100	24	160–76		61.5	98 4	18 5	Carcinoma of breast. Radical mastectomy Streptococcus hemolyticus septicemia Death March 16
	March 12	Postoperative anesthetized	4·05 p.m.	1.8	100	20	85–50	178			16.2	
		Postoperative anesthetized	6 00 p.m.	*2.9*	72	20	95–55	190		100 0 r		
5.	March 23	Basal	10·00 a.m.	2.8	78	15	138–90	187	42.2	98 8		Obstructive duodenal ulcer Posterior gastroenterostomy *Local anesthesia*
	March 25	Postoperative	3.30 p.m.	3.8	102	25	116–78	199		99 4	16.2	
	April 8	Postoperative 13th day	9 15 a.m.	2.8	74	13		178	40 9	98 6		
6	April 1	Postoperative anesthetized	2 45 p.m.	*2 7*	140	32	70–30	260	68.2	97.2	17.2	Duodenal ulcer and retroperitoneal tumor Resection of tumor and loop of jejunum. Death, April 5, peritonitis and bronchopneumonia
		Conscious	4 15 p.m.	*3.8*	112	28		262		98 4		
7	April 9	Basal	10 00 a.m.	4 4	80	11			59 0	98 6		Acute intraluminal appendicitis. Erosion of cervix, cystocele, rectocele. Perineal repair suspension of uterus, appendectomy
	April 10	Preoperative basal	7 55 a.m.	8.5	80	15	130–60	221		98 0	18 9	
	April 10	Postoperative anesthetized	11 50–12 30	2 4*	98	20	80–62	202		100 0	20 7	
	April 11	Postoperative 1st day	9 10 a.m.	3 0	96	15	100–70	227		100 7 r		
	April 13	Postoperative 3d day	9 05 a.m.	3 7	85	15	114–78	232		100.2 r	16.8	
	April 23	Postoperative 13th day	8 50 a.m.	3 9	82	14	96–62	205	56 6	98 6		
8	April 23	Preoperative basal	10 30 a.m.	7 1	96	12		211	59 6	98 4	17 0	Carcinoma of right breast. Radical mastectomy Constant intravenous postoperatively
	April 23	Postoperative anesthetized	4 25–4 50	2.3*	80	15	86–52	211	58 9	99 0	15 7	
	April 24	Postoperative 1st day	9 45 a.m.	3.8	92	15	112–64	230		99 6		
	April 28	Postoperative 5th day	8 40 a.m.	3.5	96	12	116–70	218	62.0	99 6	11 7	
9	May 18	Basal	9 30 a.m.	3 4	68	18	120–80	214		98 6		Cystocele, rectocele Perineal repair, appendectomy Constant intravenous postoperatively
	May 19	Preoperative basal	9 50 a m.	*3.9*	78	20			60.5	98 4	16.3	
	May 19	Postoperative anesthetized	1 10–1 30	2 1*	90	14	100–72	168*	60 0	99.5	16.8	
	May 20	Postoperative 1st day	10 00 a.m.	3 5	96	16	128–80	244		99 6		
	May 24	Postoperative 4th day	9 20 a.m.	3 4	78	20	148–88	262	67.2	100 0	11 9	
	May 28	Postoperative 9th day	8 55 a.m.	3.8	64	20	114–74	204	58.2	99 0	13.5	
10	June 8	Preoperative basal	8 30 a.m.	4.8	73	11	118–80	220	69 8	98 0	18 1	Cystocele, rectocele. Perineal repair Constant intravenous postoperatively
	June 8	Postoperative anesthetized	2 45–3 00	2 4*	95	16	100–72	232	68 4	98.2	15 3	
	June 9	Postoperative 1st day	9 40 a.m.	4.8	84	11	108–68			98 0		
11	June 24	8 hours postprandial	5 00 p.m.	4 9	65	16	114–68		66.5	98.2		Normal, healthy adult male. Etherization, no operation
	June 25	Pre-ether basal	11 00–12 a.m.	3 9*	63	13		248			18 9	
	June 25	During ether	2 42 p.m.	*4.1*	84	16	150–68	208				
		During ether	2 50 p.m.	*5.5*	92	15	160–60	378				
		During ether	3 00 p.m.	*5.4*	96	12	130–70					
		During ether	3 10 p.m	*3.4*	78	16	135–68	332				
		During ether	3 22 p.m.	*3 6*	82	16	125–70	320				
		Post ether anesthetized	4 10 p.m.	*3.9*	74	13	118–68				18 4	
		Post ether	4 40 p.m.	*4.8*	98	19						
	June 26	Basal next day	9 50 a.m.	4.0	76	16	120–60	248	64 7	98.6	18 9	

† Figures in italics are single estimations, those marked with * are average of triplicate estimations, others are average of duplicate estimations

‡ Body temperature is oral unless marked with letter r-rectal

§ Figures in italics are red cells in millions per cu mm , others are oxygen capacity

operatively in the other cases) (*b*) For the patient (Case 6) not studied preoperatively who went into shock during the operation, a normal cardiac output for his height and weight is assumed

The cases operated upon under ether show uniformly a reduction of cardiac output in the period of recovery from anesthesia varying from 32 to 51 per cent of the basal preoperative value, the average being 41 1 per cent This decrease per-

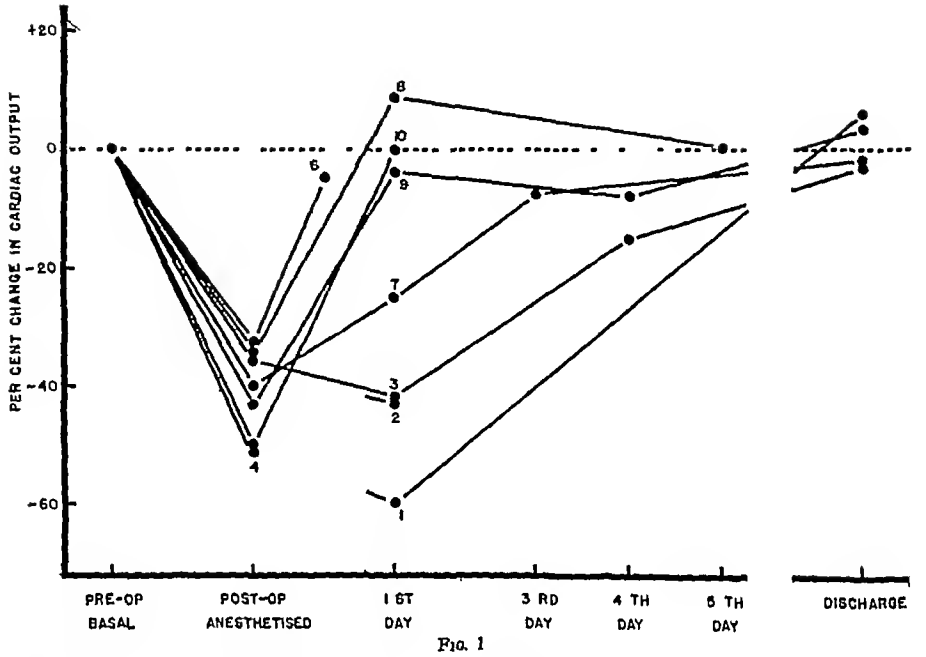


FIG. 1

PER CENT CHANGE IN CARDIAC OUTPUT PLOTTED AGAINST TIME FOR 9 PATIENTS WHO HAD ETHER ANESTHESIA

sists in some instances the following day The three patients (Cases 8, 9, and 10) who received large volumes of intravenous fluid beginning immediately after the conclusion of the first studies of postoperative cardiac output, exhibited a return of cardiac output to a normal level on the morning following operation, a level which was maintained during the succeeding studies The patient (Case 5) who had local anesthesia furnished the only instance of an elevated cardiac output immediately following operation The patient (Case 6) who had been in shock during the operation showed after recovery from shock no greater depression of output than the other cases (at the time measurements were made the patient had received two transfusions and was no longer in shock), after the third transfusion his cardiac output was normal for a man of his height and weight The patients (Cases 7 and 8) who were unduly apprehensive preoperatively showed no appreciable differences from the other patients in the postoperative period

An attempt was made to determine the cardiac output during etherization as well as during the period of recovery from anesthesia. A volunteer healthy male subject was admitted to the hospital as a patient (Case 11 Table I) He was given routine preoperative medication. Studies of cardiac output and oxygen consumption were made before, during, and after etherization without surgical procedures The preether medication (pentobarbital) made the subject so drowsy that he fell asleep repeatedly during the preliminary tests, making it difficult to obtain satisfactory basal determinations. Routine anesthesia was employed gas-oxygen for the induction process followed by ether When light third-stage anesthesia was attained the mouthpiece of the ethyl iodide apparatus was fitted to a metal airway passing behind the subject's tongue. Between the spirometer and the sampling tube for inspired air a pair of etherizing bottles in parallel was inserted. No appreciable resistance to inspiration is offered by such an arrangement regardless of the position of the valves diverting the air stream through the ether bottles This method was adopted when preliminary tests revealed that within half an hour ether vapor stored in a spirometer (painted with red lead) formed appreciable amounts of aldehydes and peroxides which render ether unfit as an anesthetic agent for humans. The anesthesia was light and quite irregu

lar, large fluctuations in pulse rate and blood pressure occurred, and the respiratory minute volume varied more than it would have done had the inspired air been charged with 2 or 3 per cent of carbon dioxide. Five determinations of cardiac output were made at 10-minute intervals during etherization, the anesthesia was discontinued, and 2 more estimations were made as the subject recovered from the etherization Studies were made again the following morning Blood samples totalling approximately 140 cc. were withdrawn for the purposes of plasma volume studies and blood gas analysis

The average of the 5 determinations of cardiac output obtained under ether is 10 per cent greater than the average for pre- and postetherization periods The latter agree closely There was no depression of cardiac output in this subject as he recovered from anesthesia The difference in depth of anesthesia between this subject and the patients who were operated upon may explain the difference in the data obtained, but the experiment was so unsatisfactory in a technical sense that no conclusions may be drawn

The oxygen consumption data (Table I) do not exhibit any consistent change

The average decrease of arterial blood pressure in the period of recovery from etherization was 31 mm Hg systolic and 11 mm diastolic (Case 11 not included)

DISCUSSION

The results of such studies of cardiac output as these may be interpreted in the manner described by Starr *et al* (18, pp 800 to 802) For the computation of a testing standard deviation, 24 pairs of duplicate estimations made on 9 subjects were available in the present study (see Table I and (9) Table II) The data were obtained entirely from hospital patients under basal conditions, pre- and postoperatively (data obtained within 60 hours after operation and anesthesia are not included in the calculations) The standard deviation is 7 9 per cent, a value which is higher than that for the 65 pairs of duplicates computed by Starr *et al*, 5 6 per cent Several factors probably contribute to this difference The patients in this study had been in the hospital for such a short time that the effect of a new environment must be considered The impending operations created many problems, for example, financial matters, apprehension of pain, discomfort, serious outcome, and so forth It is therefore not unreasonable to expect a greater variation in preoperative determinations in this group of patients than in the group studied by Starr *et al* Moreover, the modification of Starr and Gamble's technic is slightly less accurate for normals (9) Finally, the small size of the series may affect the results When the value, 7 9 per cent, is applied to the average decrease in cardiac output during the period of recovery from anesthesia, in the manner described by Starr *et al*, the probability that the difference observed is due to chance is insignificant The morphine administered preoperatively may contribute somewhat to the depression of cardiac output, but it certainly is only a minor factor in the period of recovery from anesthesia (18)

The changes in oxygen consumption in the period of recovery from ether anesthesia do not parallel the consistent decrease which is found in the cardiac output The calculated arteriovenous oxygen difference, however, shows a definite and regular increase It is not possible to estimate from these limited data the relative importance of drugs, etherization, blood loss, and operative trauma, or of changes in oxygen consumption in producing the decrease in cardiac output observed It is clear, however, that these factors, and probably others also, acting together do depress the circulation in man by a significant amount

SUMMARY AND CONCLUSIONS

Data are presented which show the cardiac output and oxygen consumption of nine patients studied before and after surgical operations, performed under ether anesthesia, by a modification of Starr and Gamble's ethyl iodide technic (Table I and Figure 1)

The average cardiac output in the period of recovery from etherization was decreased by 41 per cent of the preoperative level Return to normal required 1 to 4 days (Figure 1)

The patient receiving only local anesthesia had an elevation of cardiac output immediately after the operation (Table I)

Changes in oxygen consumption were occasionally large, but not consistent (Table I)

APPENDIX TO TABLE I

Case 1 Age 62, ♀, height 162 cm Arterial oxygen saturation Jan 12 at 4 00 p m, 98 per cent (wet heparin single estimation, under oil) Operation Jan 12 from 1 40 to 3 10 p m Con-

scious at 5 45 p.m *Medication* Jan 11, phenobarbital grains 2 at 9 p m. Jan 12, pantopon* grains ⅓ and atropine sulphate grains 1/100 at 12 30 p m. Pantopon grains ⅓ at 7 40 p.m Jan. 13, pantopon grains ⅓ at 2 00 a.m, 8.30 a m, 7 10 p m Intravenous fluid 1500 cc. 5 per cent glucose in 0 85 per cent saline from 11 a m to 1 p.m

Case 2 Age 57, ♀, height 137 cm Blood loss 0.2 liter Arterial saturation Jan 21 at 5 00 p.m 83 per cent (wet heparin single determination, under oil) Operation Jan 21, from 1 50 to 4 15 p.m Conscious at 7.30 p m *Medication* Jan 20, transfusion 500 cc. citrated blood Phenobarbital grains 2 at 9 00 p m Jan 21, pantopon grains ⅓ at 9·00 a.m Pantopon grains ⅓ and atropine sulphate grains 1/100 at 12 45 p.m , 1000 cc. 10 per cent glucose in 0 85 per cent saline 12 00 to 1·00 p m , 1500 cc. 5 per cent dextrose in 0 85 per cent saline 8 00 to 11 00 p.m Pantopon grains ⅓ at 8 15 p m , 11 15 p m Jan 22, pantopon grains ⅓ at 3·05 a m., 6 15 a m , 9 00 a.m.

Case 3 Age 49, ♀, height 173 5 cm , arterial oxygen saturation Jan 29, at 4·00 p m , 91 per cent (single determination, wet heparin, under oil) Operation Jan 29 from 1 40 to 3 20 p.m Conscious at 5·00 p m *Medication* Jan 27, phenobarbital grains 1 5 at 9·00 p m Jan 28, phenobarbital grains 2 at 9 10 p m Jan 29, pantopon grains ⅓ and atropine sulphate grains 1/100 at 12 50 p.m Morphine sulphate grains ⅙ at 11 25 p m Intravenous fluid 1500 cc. of 5 per cent glucose in 0 85 per cent saline, from 5·00 to 8·00 p.m Jan 30, morphine sulphate grains ⅙ at 3 25 a.m , 8 45 a.m , 11 50 a.m , 11 50 p m

Case 4 Age 62, ♀, height 165 cm Blood loss 0 4 liter Arterial saturation Mar 12 at 5 10 p m , 93 per cent (wet heparin, single estimation, under oil) Operation Mar 12 from 2 10 to 3 45 p m Conscious at 7 15 p m *Medication* Mar 11, phenobarbital grains 1 5 at 9·00 p.m Mar 12, pentobarbital grains 1 5 at 12 00 noon, morphine sulphate grains ⅙ and atropine sulphate grains 1/100 at 1 .30 p.m , morphine sulphate grains ⅙ at 8.35 p m

Case 5 Age 55, ♂, height 159 cm Local anesthesia Operation Mar 25 from 1 .20 to 2 41 p.m *Medication* Mar 22, morphine sulphate grains ⅙ at 3 10 p.m Atropine sulphate grains 1/100 Cevitamic acid 0 1 gram intravenously Mar 24, codein sulphate grains 1 at 3 10 a m. Phenobarbital grains 2 at 7 45 p m. Mar 25, phenobarbital grains 2 at 12 00 noon Morphine sulphate grains ⅙ at 12 10 p.m

Case 6 Age 56, ♂, height 178 cm Operation Apr 1 from 10 .20 a.m to 2 05 p.m Conscious at 3 40 p m *Medication* Mar 31, barbital grains 10 at 9 p m Apr 1, 1000 cc. 5 per cent dextrose in 0 85 per cent saline from 8 30 to 10 00 a.m Pentobarbital grains 1 5 at 8 00 a.m Morphine sulphate grains ⅙ and atropine sulphate grains 1/100 at 9 15 a.m Transfusion 600 cc. citrated blood 12 .20 p m Transfusion 600 cc. citrated blood 1 .20 p.m Transfusion 600 cc. citrated blood 3 00 p m Morphine sulphate grains ⅙ at 3 45 p m

Case 7 Age 30, ♀, height 159 cm. Blood loss 0.3 liter Arterial oxygen saturation Apr 10, at 12 noon, 92 per cent. Operation Apr 10 from 9 30 to 11 25 a m Conscious at 1 00 p.m *Medication* Apr 9, barbital grains 10 at 8 40 p m , Apr 10, pentobarbital grains 1 5 at 7 00 a.m , morphine sulphate grains ⅙, atropine sulphate grains 1/100, 8 .20 a m , morphine sulphate grains ⅙ at 1 .20, 4·00, and 7·00 p.m. 1800 cc 5 per cent dextrose in 0 85 per cent saline from 2 .30 to 4 30 p.m. Apr 11, 1000 cc. 5 per cent dextrose in 0 85 per cent saline from 10·00 to 12·00 a.m Morphine sulphate grains ⅙ at 1·00 a.m , 8 10 a.m , 2 45 p m , 8 50 p m , Apr 12, morphine sulphate grains ⅙ at 2 45 a.m., 10 .35 a m , 5 .30 p m , 10 45 p m Apr 13, morphine sulphate grains ⅙ at 11 15 p m.

Case 8 Age 38, ♀, height 157 cm , blood loss 0 5 liter, arterial oxygen saturation Apr 23 at 4 50 p m , 95 per cent. Operation Apr 23 from 2·08 to 4·05 p m. Conscious at 6 45 p m *Medication* Apr 22, pentobarbital grains 1 5 at 10 .20 p.m , Apr 23, pentobarbital grains 1 5 at 12 noon, morphine sulphate grains ⅙, atropine sulphate grains 1/100 at 1 10 p m , morphine sulphate grains ⅙ at 6 50 p m Apr 24, morphine sulphate grains ⅙ at 2 10 a m., 9 15 a.m., 12 55 p.m , 6 00 p m , 9 15 p m Apr 25, morphine sulphate grains ⅙ at 4 15 a.m., 11 10 a m., 3 .25 p m 8 40

* "Pantopon" = pantopium hydrochloricum, Hoffman Roche grains ½ contains the alkaloids in grains 1½ opium.

p m Apr 26, morphine sulphate grains ⅙ at 12 35 a m, 4 00 a m, 8 20 a m, 4 20 p m, 8 40 p m Apr 27, morphine sulphate grains ⅙ at 2 25 a m, 9 20 a m, 1 50 p m, 6 05 p m, 9 25 p m Apr 28, morphine sulphate grains ⅙ at 12 55 a m, 4 30 a m, 7 30 a m, 10 30 a m Intravenous fluids From Apr 23 at 6 p m to Apr 24 at 7 00 a m, 3 5 liters 0 85 per cent saline From Apr 24 to Apr 28, 7 to 9 liters a day

Case 9 Age 35, ♀, height 156 cm, blood loss 0 2 liter, arterial oxygen saturation May 19, at 1 47 p m, 95 per cent Operation May 19 from 11 35 a m to 12 50 p m Conscious at 2 00 p m *Medication* May 18, pentobarbital grains 1 5 at 8 00 p m May 19, pentobarbital grains 1 5 at 7 30 a m, pantopon grains ⅓ and atropine sulphate grains 1/100 at 10 50 a m Morphine sulphate grains ⅙ at 2 10 p m, 5 20 p m, 10 45 p m May 20, morphine sulphate grains ⅙ at 1 45 a m, 4 45 a m, 7 40 a m, 2 15 p m, 7 45 p m, 10 45 p m May 21, morphine sulphate grains ⅙ at 6 30 a.m, 6 45 p m May 22, morphine sulphate grains ⅙ at 7 25 a m, 3 55 p m, 8 50 p m May 23, morphine sulphate grains ⅙ at 10 35 a m, 9 00 p m May 24, morphine sulphate grains ⅙ at 10 23 a m, 12 20 p m May 25, morphine sulphate grains ⅙ at 12 05 a m May 26, pentobarbital grains 1 5 at 9 20 p m May 27, pentobarbital grains 1 5 at 9 10 p m May 28, chloral hydrate grains 20 at 2 00 a m Intravenous fluids May 19 from 3 00 p m to May 20 at 7 00 a m, 3 1 liters 0 85 per cent saline Four to 6 liters a day from May 20 to May 24

Case 10 Age 43, ♀, height 169 cm, blood loss 0 5 liter, arterial oxygen saturation June 8 at 3 00 p m, 95 per cent Operation June 8, from 12 05 p m to 2 30 p m Conscious at 3 10 p m *Medication* June 7, pentobarbital grains 1 5 at 9 00 p m June 8, pentobarbital grains 1 5 at 7 15 a m Pantopon grains ⅓ and atropine sulphate grains 1/100 at 11 15 a m Morphine sulphate grains ⅙ at 3 15 p m June 9, morphine sulphate grains ⅙ at 8 00 a m Intravenous fluids 1 9 liters saline, from 4 00 p m June 8 to 9 00 a m June 9

Case 11 Age 22, ♂, height 177 cm, arterial oxygen saturation June 25 at 4 20 p m, 95 per cent Etherization June 25 from 2 15 to 3 30 p m Conscious at 4 30 p m *Medication* June 24, pentobarbital grains 1 5 at 11 00 p m June 25, pentobarbital grains 1 5 at 7 45 a m Morphine sulphate grains ⅙ and atropine sulphate grains 1/100 at 1 45 p m Barbital grains 5 at 9 00 p m June 26, barbital grains 5 at 2 40 a m.

The advice and suggestions given by Dr Isaac Starr, and the technical assistance of Miss Margaret Rourke and Miss Virginia Dewey are gratefully acknowledged

BIBLIOGRAPHY

1 Polano, Hans, Experimentelle Untersuchungen über das Verhalten des Minutenvolumens des menschlichen Herzens bei Athernarkose, Lumbalanästhesie und nach operativen Eingriffen. Deutsche Ztschr f Chir, 1933, 239, 505

2 Broemser, P, and Ranke, O F, Ueber die Messung des Schlagvolumens des Herzens auf unblutigem Weg Ztschr f Biol, 1930, 90, 467

3 Rehn, E, Herzkraft und Kreislauf bei mittelbarer und unmittelbarer chirurgischer Belastung Klin. Wchnschr, 1936, 15, 1625

4 Bazett, H C, Cotton, F S, Laplace, L B, and Scott, J C, The calculation of cardiac output and effective peripheral resistance from blood pressure measurements with an appendix on the size of the aorta in man. Am J Physiol, 1935, 113, 312.

5 Altschule, M D, and Volk, M C, Therapeutic effect of total ablation of normal thyroid on congestive failure and angina pectoris XVIII The cardiac output following total thyroidectomy in patients with and without congestive heart failure, with a comparison of results obtained with the acetylene and ethyl iodide methods Arch. Int. Med, 1936, 58, 32

6 Johnson, G S, and Blalock, A, Experimental shock. XII A study of the effects of hemorrhage, of trauma to muscles, of trauma to the intestines, of burns, and of histamine on the cardiac output and on blood pressure of dogs Arch. Surg, 1931, 23, 855

7 Blalock, A, Cardiac output in the dog during ether anesthesia I The effect of ether anesthesia on the cardiac output. Arch Surg, 1927, 14, 732

8 Starr, I, Jr, and Gamble, C J, An improved method for the determination of cardiac output in man by means of ethyl iodide. Am J Physiol, 1928, 87, 450

9 Snyder, J C, The determination of the cardiac output in man at brief intervals by a modification of the ethyl iodide method J Clin. Invest., 1938, 17, 563

10 Cool, R D, Determination of small amounts of ethyl iodide. J Biol. Chem, 1932, 97, 47

11 Cool, R D, Gamble, C J, and Starr, I, Jr, The solubility of ethyl iodide in human blood and its

correlation with the erythrocyte count. J Biol. Chem., 1934 105, 97

12. Van Slyke, D D, and Neill J M., The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I J Biol. Chem., 1924, 61, 523

13 Shaw J L., and Downing V., The determination of oxygen in blood in the presence of ether by a modification of the Van Slyke-Neill technique. J Biol Chem. 1935 109, 405

14 Starr, I., Jr., Donal J S., Margolies, A., Shaw R., Collins, L. H., and Gamble, C. J., Studies of the heart and circulation in disease estimations of basal cardiac output, metabolism heart size, and blood pressure in 235 subjects. J Clio Invest., 1934, 13, 561

15 Snyder, J C., A note on the use of the Haldane apparatus for the analysis of gases containing ether vapor J Biol. Chem., 1937 122, 21

16. Gatch, W D and Little, W D., Amount of blood lost during some of the more common operations Preliminary report. J A. M A., 1924, 83, 1075

17 Stewart, John D., and Rourke, G M., Changes in blood and interstitial fluid resulting from surgical operation and ether anesthesia. J Clin. Invest., 1938 17, 413

18 Starr, I Gamble, C. J., Margolies A., Donal, J S, Jr Joseph, N and Eagle, E., A clinical study of the action of 10 commonly used drugs on cardiac output, work, and size, on respiration, on metabolic rate, and on the electrocardiogram. J Clin. Invest., 1937 16, 799

MEASUREMENTS OF THE CIRCULATION IN CONSTRICTIVE PERICARDITIS BEFORE AND AFTER RESECTION OF THE PERICARDIUM [1]

By HAROLD J STEWART, GEORGE J HEUER, JOHN E. DEITRICK, NORMAN F CRANE, ROBERT F WATSON AND CHARLES H. WHEELER

(From the New York Hospital Departments of Medicine and Surgery Cornell University Medical College New York City)

(Received for publication April I, 1938)

Experience is showing that chronic constrictive pericarditis is not an uncommon syndrome. Attention has been directed to it again in recent years Its recognition has been facilitated by White's (1) historical resumé and analysis of its clinical features It is of therapeutic importance to recognize this syndrome for it is a cardiac affection that lends itself to surgical treatment as the experience of Churchill (2), Beck and Griswold (3), Blalock (4), and of Stewart and Heuer (5), as well as others, has demonstrated The clinical manifestations of this disease have been very well described, the pathological physiology of the circulation has not, however, been sufficiently explored

In the last two and a half years, we have observed 9 patients suffering from chronic constrictive pericarditis, and in six of these part of the pericardium has been resected by Dr George J Heuer [2] In them, studies of the circulation have been made before as well as after partial pericardiectomy One clinical group does not have the opportunity to see large numbers of these patients in a short time, and for this reason this paper records our studies of the circulation in this situation, together with a statement of our experience with surgical treatment.

PLAN OF STUDY

All patients were admitted to the hospital and remained in bed. The daily fluid intake was limited to 1200 cc., and the salt to 2.0 grams A high protein diet was given

Studies of the circulation were made when the patients first came under observation and before we instituted drug therapy Diuretics were then administered and studies were repeated, this time when they were in the best state it was possible for them to attain. Resection of the pericardium was then done. As soon after operation as the patients were able to participate, observations were repeated as well as later when changes in the clinical state occurred. The patients were discharged from the hospital free of excess fluid and followed in the Cardiac Outpatient Clinic, from which they were readmitted to the hospital at intervals for repetition of the studies of the circulation.

METHODS

All observations were made in the morning while the patients were in a basal metabolic state. Measurements of the cardiac output were made by the acetylene method, three samples of gas being taken as first recommended by Grollman (6), and by Grollman, Friedman, Clark, and Harrison (7) During this measurement the patients were sitting in a steamer chair (angle 135 degrees) They were trained beforehand to carry out the procedures. While the patient was at rest, the cardiac rate was counted at intervals of five minutes. At the end of one half hour the acetylene-air-oxygen mixture was rebreathed. Three samples of gas were taken during each rebreathing period for estimation of the arteriovenous oxygen difference. The first sample was taken after rebreathing 10 to 12 times in 20 seconds the second after 2 to 3 breaths more, and the third after 2 to 3 additional breaths. All three samples were usually obtained before the end of 30 seconds Samples were taken during expiration. Two to three periods of rebreathing were carried out on each patient. Shortly afterward the oxygen consumption was measured with a Benedict Roth spirometer After a short panse, the vital capacity was measured, and height and weight recorded. In succession, sufficient time being allowed between each procedure for the patient to return to a basal metabolic state, an electrocardiogram was taken, the arm to tongue circulation time recorded, the venous pressure estimated and the blood pressure measured, finally an x ray photograph of the heart was made at a distance of two meters

The arm to tongue circulation time was estimated by the use of decholin (8) Five cc. of a 20 per cent solution were injected rapidly (1 to 2 seconds) through an 18-gauge needle into an antecubital vein while the patient was lying quietly in the supine position. This was re-

[1] An abstract of these studies was read before the Association of American Physicians Atlantic City May 5 1937

[2] Since this paper went to press the pericardium was resected from another patient, making seven cases in all (5)

peated in one and one-half minutes after the response to the first test had been elicited The time was recorded from the beginning of the injection until the patient perceived the bitter taste.

The venous pressure was measured by the direct method (9), using a large antecubital vein, the arm being placed on a level with the right auricle. Normal pressures by this method range from 4 0 to 10 0 cm of saline. The antecubital vein of one arm was reserved for the injection of decholin and of the other arm for the measurement of venous pressure In subsequent measurements the vein was entered at the site first punctured.

X-ray photographs of the heart were taken with the patient in the standing position, in full inspiration, at a distance of two meters [3] Measurements of the cardiac area were carried out by the technique of Levy (10) and estimations of volume were made as recommended by Bardeen (11) Special x-ray exposures in the anterioposterior as well as in the lateral positions were taken for the detection of calcification Examination under the fluoroscope was also carried out. In certain patients photographs of the eyegrounds were made for definition of the vessels Infra-red photographs of the patients were taken to record the state of the peripheral veins The patients assumed as nearly as possible exactly the same position for each observation in order to assure uniformity from this point of view In addition, each procedure in the observation was carried out by the same investigator

The six patients who were operated upon and in whom observations were made both before and after partial pericardiectomy form the subject of this paper

RESULTS

The data are recorded in Tables I and II and certain of the data are summarized in Figure 1

The arteriovenous oxygen difference before operation was increased in all except one (Case 6, 59 7), the range being from 71 5 to 88 6 cc After operation when the patients were in their best state, it decreased in all patients and only one fell outside of the normal range, which was then 51 4 to 68 7 cc

The cardiac output per minute and cardiac index [4] were decreased in all except one patient (Case 6, 2 16 liters), the range of the index being 1 35 to 1 82 liters After operation it increased and ranged between 1 80 to 2 72 liters, and was below normal in only one (Case 4)

The stroke volume was decreased and the range was from 20 to 42 cc per beat After operation it increased and ranged from 33 to 50 cc. per beat.

The venous pressure was elevated in every case, the range being 17 9 to 24 0 cm After operation it fell and when the patients were in their best state the range was 8 3 to 16 7 cm

The arm to tongue circulation time ranged from 13 5 to 29 8 seconds before operation, in short, it was prolonged After operation the range was 7 3 to 17 1 seconds when the patients were in their best state

There was no consistent behavior of the heart rate In certain patients it was elevated before operation and slowed afterward, and in others the reverse happened The basal metabolic rate was not altered significantly in this syndrome, nor was it changed by operation

The vital capacity before operation was not lowered if the pleural cavities were free of fluid In certain patients it decreased and in others it increased after operation Decrease after operation was in part due to the flexible thoracic cage resulting from removal of the ribs

Infra-red photographs revealed marked distention of and increase in the number and caliber of the venous channels before operation As improvement occurred after operation there was progressive decrease in their number and caliber (Figure 2)

CLINICAL COURSE OF PATIENTS

After operation there were 3 trends (*1*) Clinical improvement was rapid and striking, and associated with this were changes toward normal of the measurements of the circulation (Tables I and II, Figure 1) In two patients "cure" was a matter of months (Cases 1 and 2) (*2*) In one (Case 3), clinical improvement was slow and gradual, to cure in approximately 1 year after operation (Tables I and II), in her, the measurements of the circulation showed gradual changes (*3*) Three patients improved gradually after operation, their condition has now become stationary (Cases 4, 5, and 6) They are better than before the operation and are ambulatory, and there have been changes in the circulation toward normal (Tables I and II)

[3] The authors are deeply indebted to the X-ray Department of the New York Hospital for their cooperation in this investigation

[4] Cardiac index = liters per square meter per minute.

TABLE I

Data relating to 6 patients suffering from chronic constrictive pericarditis

Case number; Initials; History number; Age; and Sex	Date	Height	Weight	Oxygen consumption	Arteriovenous oxygen difference	Cardiac output	Cardiac output	Heart rate	Stroke volume	Arterial pressure	Circulation time	Venous pressure	Vital capacity	Diglalls	Other drugs	Evidence of congestive heart failure[a]							Red blood count	Hemoglobin	Parenchymal pulse[b]
																Dyspnea	Cyanosis	Rales	Liver	Ascites	Edema	Pleural effusion			
		cm.	kgm.	c.c. per min-ute	cc.	liters per min-ute	liters per sq. m. per minute	per min-ute	cc.	mm. Hg	seconds	cm.	cc.	grams									millions	per cent	
Case 1 A. B., No. 25323[1] 15 years ♀	May 18, 1936	150.5	40.9	160	71.7	2.23	1.70	112	20	93/84	15.4	24.0	1900	0	[c]	0	0	0	+	++	0	0	3.8	76	++
	June 2, 1936	151.0	37.0	141	66.5	2.13	1.70	75	27	82/70	15.5	18.5	1850	0	Aminophyllin 0.1 gm. t.i.d.	0	0	0	+	++	0	0	4.3	82	++
	Operation on June 5, 1936																								
	June 8, 1936											17.5											4.8	80	
	June 23, 1936	150 7	34.7	139	52.1	2.67	2.20	84	32	85/60	10 4	9.6	1350	0		0	0	0	+	++	0	0			0
	June 29, 1936	151.0	34.7	143	48.5	3.00	2.42	88	34	88/55	10 4	11.4	1500	0		0	0	0	+	++	0	0	4.1	92	
	Oct. 20 1936	151.0	39.7	153	43.2	3.53	2.70	90	39	90/68	10.1	7.8	1800	0		0	0	0	+	+	0	0	4.9	100	
	Apr 6, 1937	151.0	44.9	193	51.1	3.75	2.73	93	41	100/60	7.5	8.3	1800	0		0	0	0	+	+	0	0			0
Case 2 W. M., No. 103609 38 years ♂	Sept. 10, 1935	172.5	58.7	199	51.9	3.83	2.28	96	40	104/70	21.3	13.0	2200	0	Theocalcin 1.5 gm. t.i.d.	+	+	0	+	+	0	0	5.8	113	++
	Sept. 17 1935	172.5	58.3	199	52.5	3.18	1.87	88	36	94/64	25.2	14.3	2100	0	[d]	+	+	0	+	+	0	0			+
	Sept. 21, 1935	172.5	58.7	199	50.6	3.00	1.73	88	34	94/60	22.0	14.8	2000	0		+	+	0	+	+	0	0			+
	Sept. 26, 1935	172.5	58.0	209	53.0	3.88	2.28	86	45	98/68	20.0	11.0	2500	0	Theocalcin 1.5 gm. t.i.d.	+	+	0	+	+	0	0			+
	Sept. 30 1935	172.5	57.3	214	54.5	3.93	2.28	84	47	102/64	19 4	7.3	2800	1.5 on Sept. 29 1935	Theocalcin 1.5 gm. t.i.d.	+	+	0	+	+	0	0			+
	Oct. 7 1935										21.0	6.3			Theocalcin 1.0 gm. t.i.d.										
	Oct. 12, 1935										22.2	15.7			Theocalcin 1.0 gm. t.i.d.										
	Nov 1, 1935	172.4	60.3	222	71.0	3.13	1.23	86	36	96/65	21.2	12.5	3200	0	Theocalcin 1.0 gm. t.i.d.	+	+	0	+	+	0	0			+
	Nov 11, 1936	172.5	58.5	203	74.2	2.74	1.60	70	39	116/90	18.4 (other	21.0	1800	0	[e]	+	+	0	+	+	0	0	5.0	92	+
	Operation on Nov 25, 1935																								
	Dec. 5, 1936										18.8	13.5	(not heard)	0											
	Dec. 11, 1936	172.0	54.7	187	58.3	3.51	2.14	72	49	95/63	20.2	14.3	1800	0		±	+	+	+	+	0	0	5.1	102	
	Dec. 29, 1936	172.5	57.2	199	53.4	3.73	2.25	72	52	105/78	21.8	13.8	2500	0		±	+	↑	+	+	0	0	5.3	106	+
	Apr 1 1937	172.0	53.5	193	53.5	3.59	2.19	80	45	93/75	16.7	7.1	1900	0		+	0	0	+	+	0	0			+
	Jan. 28, 1938										19.3	9.3													
Case 3 A. R. Ca. 91848 30 years ♂	Dec. 20, 1935	154.7	42.7	180	68.6	2.14	1.55	74	29	92/66	20.7	20.8	1500	0.1 b.i.d.[10]	A.C.[11] 1.0 gm. t.i.d.	0	+	+	+	+	0	0	4.3	90	+
	Jan. 27 1936	154.0	45.9	201	81.3	3.45	1.76	100	35	92/70	15.0	21.5	1600	0[13]	A.C. 1.0 gm. t.i.d.[12]	0	+	↓	+	+	0	0			+
	Operation on Jan. 23, 1936																								
	Jan. 28, 1936							120			13.7	20.3													
	Feb. 4, 1936							120		110/83	15.5	22.0	1900	0		±	+	0	+	+	0	0	3.9	77	+
	Feb. 13, 1936	153.0	47.7	156	65 0	2.19	1.52	110	20	100/75	10.9	27.5	900	1.5 on Feb. 14, 1936		0	+	0	+	+	0	0			+
	Feb. 15, 1936	153.5	46.8	184	65.7	2.78	1.94	96	29	99/64	10.2	22.7		0.1 b.i.d.											
	Feb. 17, 1936	153.5	47.5	195	72.5	2.65	1.84	84	32	105/75	8.0	22.0	950	0		0	+	+	+	+	0	0			+
	Mar. 27 1936	154.0	48.4	187	71.5	2.54	1.70	88	29	108/72	9.0	19.2	1400	0[14]		0	+	+	+	+	0	0	4.4	82	+
	May 9, 1936	154.5	49.9	197	57.2	3.44	2.24	80	43	105/78	14.4	9.8	2000	0		0	+	↑	+	+	0	0	4.3	84	+
	Mar. 18, 1937	153.5	51.5	191	54.9	3.35	2.25	80	42	80/50	14.0	8.5	2500	0		0	+	+	+	0	0	0	4.5	100	+
	Nov 30, 1937	154.5	50.5	178	70.3	2.70	1.84	88	31	100/60	17.6	8.0	2800	0		0	0	0	+	0	0	0	4.0	82	0
	Dec. 16, 1937	154.5	48.7	161	63.4	2.90	2.00	80	36	99/65	12.4	4.8	2850	0		0	0	0	±	0	0	0	4 1	87	0
Case 4 Mrs. 1867/43 Age	Nov 19, 1936	173.0	61.3	228	81.3	2.81	1.63	96	29	93/70	24.0	14.5	1400	0		0	+	+	+	+	+	0	R.C.	5.7	101	+
	Dec. 8, 1936	174.0	61.5	205	81.7	3.51	1.44	96	26	100/70	24.0	29.4	1400	0		0	+	+	+	+	+	0	R.C.	5.2	100	+
	Operation on Dec 21, 1936																								
	Jan. 3, 1937	173.5	61.4	218	76.6	2.84	1.57	102	28	105/80	20.0	22.5	1300	0	A.C. 1.0 gm. t.i.d.	0	+	+	+	+	+	0	R.C.	5.0	100	+
	Jan 23, 1937										22.1	23.0		0	A.C. 1.0[15] gm. t.i.d.										
	Mar 29, 1937	173.5	59.4	207	63.7	3.01	1.80	90	33	90/70	17.1	16.7	2000	0	A.C. 1.0 gm. t.i.d.	0	0	0	+	±	0	+	R.C.	5.6	110	+
	[illegible] 1938[16]	171.5	55.1	232				88		110/80		21.5	1700	0	Urea, 10.0 gm. t.i.d.								R.C.		+

TABLE I—*Continued*

Case number[1] Initials History number Age and Sex	Date	Height	Weight	Oxygen consumption	Arteriovenous oxygen difference	Cardiac output	Cardiac output	Heart rate	Stroke volume	Arterial pressure	Circulation time	Venous pressure	Vital capacity	Digitalis	Other drugs	Evidence of congestive heart failure[2]							Red blood count	Hemoglobin	Paradoxical pulse[3]
																Dyspnea	Cyanosis	Rales	Liver	Ascites	Edema	Pleural effusion			
		cm.	kgm.	cc. per minute	cc.	liters per minute	liters per sq m per minute	per minute	cc.	mm. Hg	seconds	cm	cc.	grams									millions	per cent[4]	
Case 5	Aug. 21, 1936	156 0	64.0	184	83 1	2.22	1 35	66	34	122–116/78	18.6	17 9	1650	0 1 q.d.	None[17]	0	+	+	+	± +	+	Rt.	5 1	122	+
P A.,	Sept. 22, 1936	156 0	57 0	191	71.5	2 69	1 71	78	34	128/72	18.4	13 8	2280	0 1 q.d.		0	+	0	+	±	+	Rt. ↑	5 3	104	+
No 141257	Operation on Sept. 28, 1936																								
44 years	Nov 12, 1936										13 3	16 1		0 1 b.i.d.	A C 1 0 gm. t.i.d.										
♀	Dec. 10, 1936	154.7	43 9	172	61.8	2 78	2.01	64	43	110/70	11.2	11.3	1200	0 1 b.i.d.	A.C 1 0 gm. t.i.d.	0	+ ↓	0	+	0	+ ↑	Rt. ↑	4.0	78	+
	Mar 31 1937	155 5	57 7	209	75.3	2 78	1 76	80	35	128/68	16.7	19 8	1400	0 1 b.i.d	Aminophyllin 0 1 gm. t.i.d.	+	+ ↓	+	+	0	+ ↓	Rt. ↓	4.3	96	+
	Feb. 1, 1938	154.8	58.1	203	64.2	3 16	2 02	84	38	100/80	19 2	15 3	750	0 15 q.d.	Urea, 15 0 gm. t.i.d.	+	+	+	+	+ ?	+	Rt.			
Case 6	May 26, 1937	168.5	104.3	278	59 7	4.66	2.20	112	42	130/75	12.5	20 1	2000	0	0	+	+	+	+	+	+	0	5.5	110	+
J S.,	June 15 1937	166 0	93.2	251	58.3	4.31	2.16	88	49	118/70	13.5	12 7	2300	0	Theocalcin 1 5 gm t.i.d.	0	0	+ ↓	+ ↓	+ ↓	+	0			
No 169168	Operation on June 17, 1937																								
38 years	July 1 1937	165 0	88.0	234				82		110/88	10 2	11 1	1800	0	0	0	+	0	+	+	+ ↓	0	4.8	90	+
♀	Aug. 6, 1937										9 0	10.2													
	Aug. 27, 1937	164.5	72 0	224	61 0	3 67	2.05	78	47	95/65	16 7	12.9	3350	0	A.C. 1 0 gm. t.i.d.	0	0	0	+ +	±	+ ↓	0	4 9	86	+
													3450	0	[18]	0	0	0	+ ↓	0	0	0	5 9	96	+
	Nov 19, 1937	165.8	72 4	222	65.5	3.39	1.88	74	46	120/80	18.0	10 7	3600	0	A C 1 0 gm. t.i.d. and	0	0	0	+	0	0	0			+
	Nov 26, 1937	164.8	73.8	220				74		85/68	19.5	8.7			theocalcin 1 0 gm. t.i.d.										
	Jan. 28, 1938	165 3	86 9	220	56.5	3.89	2 00	78	50	105/75	21 4	8.3	2900	0	A C 1 0 gm. t.i.d.	±	±	0	+	±	+	0			+

[1] The rhythm of the heart was normal sinus rhythm in all the patients except P A , Case 5

[2] The serum protein values were repeatedly shown to lie within normal limits in all the patients except P A , Case 5, and J S , Case 6, in whom the total protein repeatedly remained between 4 0 and 5 0 grams per cent

[3] 0, +, ±, ↑, ↓, = absent, present, doubtful, increased, decreased, respectively

[4] 14 5 grams hemoglobin equivalent to 100 per cent

[5] +, 0, ± = present, absent, questionable, respectively

[6] Mercupurin, 1 0 cc., was given intravenously on May 17, 1936

[7] No theocalcin had been given since September 10, 1935

[8] No theocalcin had been given since November 6, 1935

[9] On March 18, 1937, the patient was pregnant On November 30, 1937, the patient was pregnant Therapeutic abortion by means of miniature Caesarean Section was performed on December 3, 1937

[10] On the days when special studies of the circulation were made, maintenance doses of digitalis, or doses of other drugs, were not given to any of the patients until after the studies had been completed

[11] A C = ammonium chloride

[12] No digitalis had been given since December 28, 1935

[13] Mercupurin, 2 0 cc , was given intravenously on January 26, 1936

[14] No digitalis had been given since March 5, 1936

[15] "Resting" and not "basal" measurements

[16] Mercupurin, 2 0 cc , was given intravenously on February 1, 1937

[17] Mercupurin, 2 0 cc , was given intravenously on September 21, 1936 Theocalcin, 1 5 grams t i d , and ammonium chloride, 1 0 gram t i d , were also being given

[18] Mercupurin, 2 0 cc., was given intravenously on November 17, 1937 Theocalcin, 1 0 gram t i d , and ammonium chloride, 1 0 gram t i d , were also being given

TABLE II

Additional data relating to 6 cases of chronic constrictive pericarditis *

Case number-Initials; and History number	Time	Duration of disease†	Etiology	Heart size	Calcification of pericardium	Fluoroscopy of heart	Electrocardiogram	Result	Time since operation‡
Case 1 A. B., Number 28333	Before operation	5 years 6 months	unk.	Very small	Present	Very small pulsations	Sl. R.A.D. $QRS_{1,2,3}$ and $T_{1\ 2,3}$ low ampl. $T_{3,3}$ cove shaped. Axis shift 37°		
	After operation			Larger	Present	Very good pulsations	Axis shift 22° No other change	Cured	1 year 9 months
Case 2 W M Number 103699	Before operation	1 year 4 months	unk.	Large	0	Sl. of lt. side, none of rt. side. No downward shift, sl. lt. shift	R.A.D. $QRS_{1,2,3}$ low ampl. $T_{1,2,3}$ neg. Axis shift 0°		
	After operation			Smaller	0	Good pulsations	R.A.D $QRS_{1,2,3}$ still low ampl. $T_{1\ 2,3}$ incr ampl. Axis shift 9°	Cured	1 year 4 months
Case 3 A. R., Number 91648	Before operation	4 months	tbc.	Sl. enlarged	0	Decr pulsations of rt. aur and rt. vent.	Sl. R.A.D $QRS_{1\ 2,3}$ low ampl. $T_{1,2,3}$ low ampl. Axis shift 11		
	After operation			Smaller	0	Excellent pulsations	No change. Axis shift 6°	Cured	2 years 2 months
Case 4 J McC., Number 123763	Before operation	7 months	unk.	Large	0	No motion lower ⅓ of heart	Sl. L.A.D. $QRS_{1\ 2,3}$ low ampl. $T_{1,2}$ cove shaped. Axis shift 11		
	After operation			No change in size, but shape changed	0	Incr motion but none of rt. vent.	No axis deviation. Incr ampl. of $QRS_{1,2,3}$. Axis shift 18°	Improved	1 year 3 months
Case 5 P A., Number 141257	Before operation	§	unk.	Large	Present	Decr pulsations of rt. aur	Sl. R.A.D $QRS_{1,2,3}$ low ampl. $T_{1,2,3}$ diphasic. Axis shift 38		
	After operation			Not much change	Present	Incr along lt. but almost none of rt. aur and rt. vent.	No change. Axis shift 30°	Improved	1 year 8 months
Case 6 J. H., Number 169165	Before operation	6 years	unk.	Small	Not seen in x-ray	Sl pulsations of lt. vent.	Sl. L.A.D $QRS_{1,2}$ low ampl. Low ampl. of T_2. Axis shift 0°		
	After operation			Larger	Present in micro. sec. of pericard.	Incr pulsations	Sl. L.A.D, $QRS_{1,2}$ sl. incr in ampl. $T_{1,2}$ incr in ampl. Axis shift 30°	Improved	8 months

* In this Table the following abbreviations are used
unk. = unknown
tbc. = tuberculosis
sl. = slight
lt rt. = left right respectively
incr., decr = increased, decreased, respectively
ampl = amplitude
neg = negative
R.A D , L.A D = right and left axis deviation respectively
aur vent = auricle ventricle respectively
micro sec. of pericard = microscopic sections of pericardium
† As estimated from evaluation of patients' history
‡ As of March 1938
§ Edema of ankles was noted 10 years before admission Symptoms were first noted 2 months before admission

DISCUSSION

Our observations show that chronic constrictive pericarditis is characterized by decreased cardiac output per minute and per beat, rise in venous pressure, slowing of the velocity of blood flow, and engorgment of the venous vascular bed Fluoroscopic examination shows decreased contraction of the heart chambers, and fixation of the heart may be observed Clinical improvement after operation was associated with changes in all these functions toward normal There appear to be two essential defects in this syndrome, namely, (*1*) obstruction to the entrance of blood into the chambers of the heart resulting in decreased filling, and (*2*) interference with contraction of the heart. There is evidence for the first in (*a*) the decreased dilatation of the heart in diastole under fluoroscopic examination and at operation and (*b*) the observation at operation of the thickened pericardium which was not capable of much distention and may have been calcified (*c*) Infra red photographs revealed distention of the peripheral veins (*d*) The elevated venous pressure is evidence that there is ample

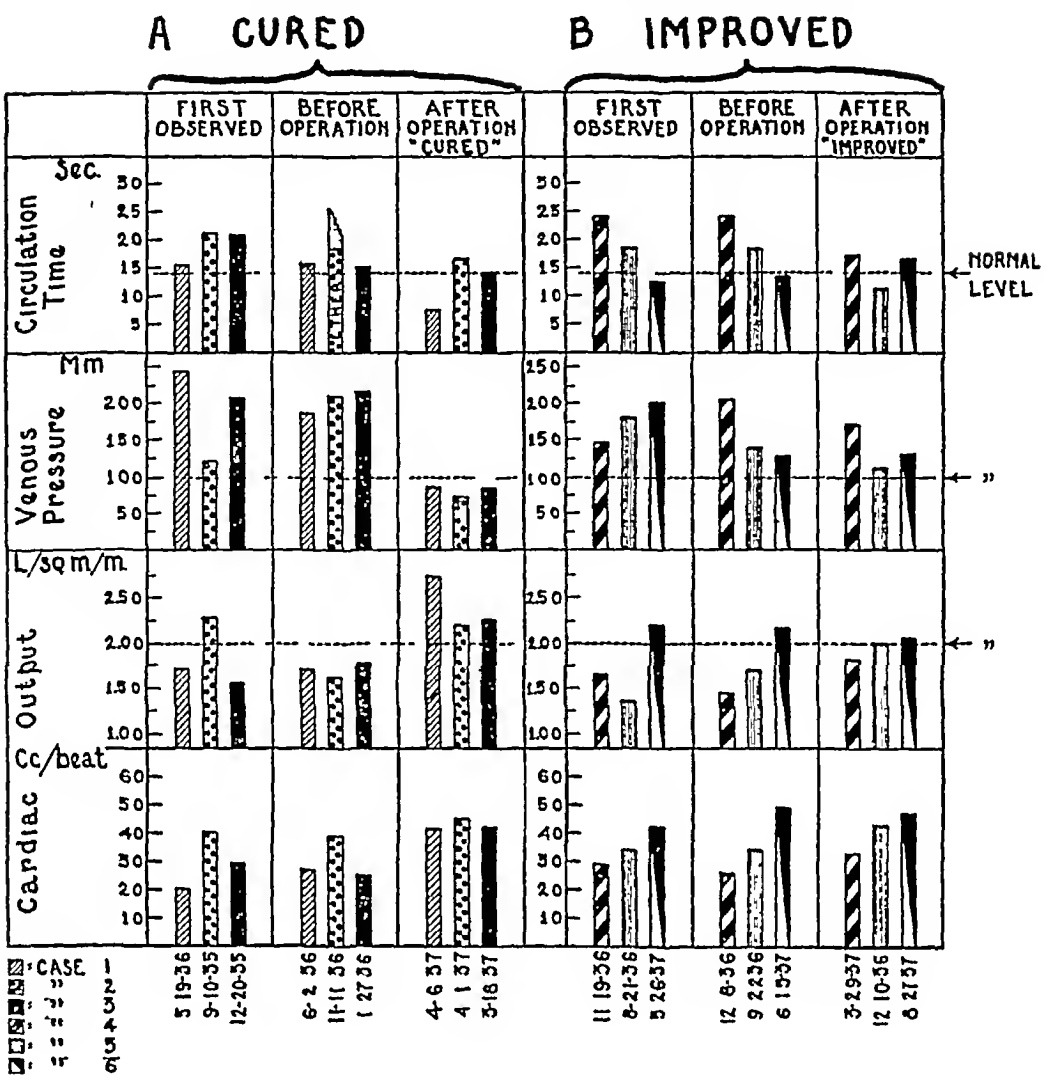


FIG 1

Data relating to 3 patients "cured" by operation (A) and 3 "improved" by operation (B)

blood available for the heart With respect to contraction, impairment is inferred from the (*a*) decreased extent of contraction on fluoroscopic examination and at operation, as well as from (*b*) the examination of the thickened unyielding pericardium incapable of much change during contraction These two defects result in decrease in cardiac output, per beat and per minute, and piling up of blood on the venous side, accounting for increase in venous pressure and slowing of the velocity of blood flow The heart may be unusually small or not much enlarged [5] Removal of the pericardium results in alteration of these two defects, in short, in removing obstruction to blood entering the heart, allowing the heart to stretch in diastole, and increase in extent of contraction, these account for changes in the circulation which have been recorded after operation Parallel with improvement in circulation after operation, clinical improvement occurs

These observations with respect to cardiac output and venous pressure are in accord with those reported by Burwell and his associates (12, 13), and by Beck and Cushing (14)

The delay in improvement of certain patients after operation may be due in part to dilatation of the region of the heart from which the pericardium has been resected, and in part to ob-

[5] The cardiac silhouette is made up of cardiac shadow plus the shadow of the thickened pericardium.

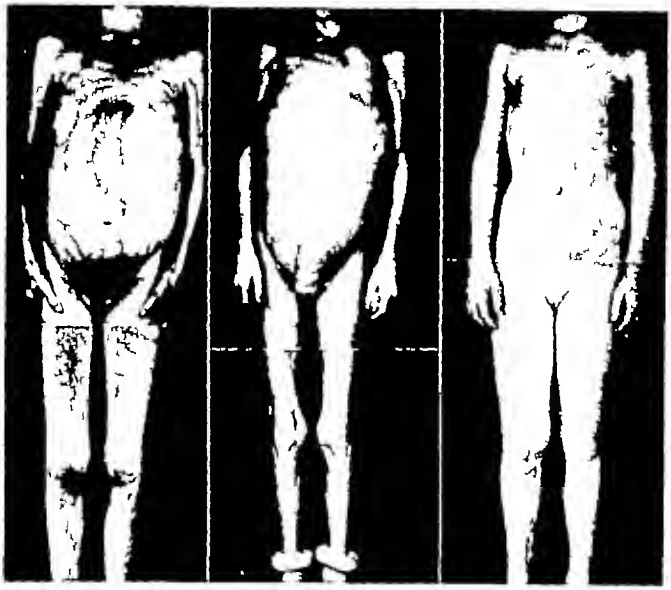


FIG 2

Infra red photographs of A.B (Case 1) in whom operation was followed by cure. Photograph A was made on May 20 1936 B on June 29 1936 20 days after pericardiectomy and C on April 4 1937 approximately 10 months after operation Attention is directed to the decrease in venous channels to the patient's growth and development and to change in flare of the ribs with the elimination of the chronic ascites from which the patient had suffered five and a half years

struction not having been sufficiently relieved At operation the heart bulged through the window which was made in the pericardium and undue stretching of this muscle may have resulted It may require time for this muscle to regain 'tone'

Three patients (Cases 2 3, and 4) were observed from the stage of pericarditis with effusion through the stage of constriction In them signs of obstruction disappeared with absorption of the pericardial fluid but increased rapidly when formation of adhesions and constriction began and was associated with rapid parallel rise in venous pressure and circulation time and decrease in cardiac output

The use of digitalis appears to be contraindicated except under certain circumstances Stewart and his associates have shown that digitalis decreases cardiac size (15 16 17) and increases the extent of ventricular contraction (18 19, 20) In the presence of constrictive pericarditis the size of the heart may already be restricted and the cavities small and further decrease in its size may not be beneficial but may increase the obstruction On the other hand the heart is probably contracting as fully as possible while it is attached to the unyielding thickened pericardium One patient (Case 5) exhibiting auricular fibrillation was under the influence of digitalis when she came under observation Its use seemed essential to maintain a slow heart rate, and it did not appear justifiable to withdraw the drug To one patient (Case 3) exhibiting normal sinus rhythm the drug was given *after* resection of the pericardium In this instance increase in cardiac output and fall in venous pressure occurred effects which were to be expected (15 16 17) (Table I) Change in size of the heart could not be estimated because of the presence of fluid

in the right pleural cavity. Digitalis was given to another patient (Case 2) exhibiting normal sinus rhythm. We were unable to state the stage of the pericardial lesion at the time of exhibition of the drug, it was probably in the stage of absorption of pericardial fluid and early formation of adhesions. The patient was taking theocalcin at the time the first observations were made (Table I). When the drug was discontinued there was rise in venous pressure, slowing of circulation time and decrease in cardiac output. When it was given again the venous pressure fell, the circulation time decreased, and cardiac output increased, and all the measurements were in the normal range. We were unable to evaluate the effects of giving digitalis, 1.8 grams, on September 29, 1935. The control measurements were made 4 days beforehand and spontaneous changes which would invalidate the comparison may have occurred in the circulation in this interval. The effects of theocalcin can, however, be evaluated and were similar to those already recorded by Stewart and Cohn (15).

Case 6 calls for comment. Except for the elevated venous pressure, the measurements of the circulation were in the normal range before operation. She improved before operation by medical treatment and the use of drugs. She was able apparently to maintain at rest in bed a normal cardiac output per minute and cardiac index and approximately normal output per beat in spite of the very thickened adherent pericardium. Operation resulted only in moderate clinical improvement, and the venous pressure fell to normal. Subtotal thyroidectomy had been performed on this patient early in her illness 6 years before, when it was suspected that heart failure was caused by hyperthyroidism. Whether this accounted for the deviation of the patient from the pattern of the other patients, we are unable to state. The circulation was not maintained at the expense of an elevated heart rate, nor was there anemia (21). The plasma protein in this patient remained low although she was given a high protein diet. Before operation, diuretics were ineffectual in this patient. After operation, however, urine outputs of 5 liters a day resulted from 2.0 cc. injections of mercupurin, and 16 kgm. of weight were lost in 6 weeks (Table I).

The partition of the circulation time was studied in 4 patients (Table III). The arm to tongue circulation time was measured by the use of decholin (D), the arm to lung time by the injection of ether (E) (22), and the lung to respiratory center by the inhalation of carbon dioxide (C) (23). D minus E should approximate the value of C, which was found to be the case when observations were made (Table III). The ether arm to lung circulation time was in the normal range in Cases 2, 5, and 6, and prolonged in Case 4. The lung to respiratory center time was prolonged in Cases 2, 5, and 6, and normal in Case 4. In short, no uniformity was apparent.

TABLE III

Data relating to partition of circulation time in 4 cases

Patient	Date	Venous pressure	Circulation time			
			Arm to tongue Decholin (D)	Arm to lung Ether (E)	Lung to respiratory center CO (C)	D-E should equal (C)
		cm	*seconds*	*seconds*	*seconds*	*seconds*
Case 2	Jan. 28, 1938	9.2	19.2	5.5	15.0	13.7
Case 4	Jan. 29, 1938	18.8	21.5	13.5	9.5	8.0
Case 5	Feb. 1, 1938	15.3	19.2	6.0	13.5	13.2
Case 6	Feb. 28, 1938	15.5	21.4	8.3	12.0	13.1

The effect of pregnancy was observed in one patient (Case 3). Observations made on November 30, 1937 (Table I) when she was 3 months pregnant revealed increase in arteriovenous oxygen difference, decrease in cardiac output per minute and per beat, and in cardiac index, and lengthening of the circulation time, not only on comparison of them with the measurements on March 18, 1937, before pregnancy occurred, but also when restoration to normal was found on December 16, 1937, 13 days after therapeutic abortion.

SUMMARY AND CONCLUSION

Chronic constrictive pericarditis is usually associated with decrease in cardiac output per minute and per beat, and decrease in the cardiac index. The venous pressure is elevated and the circulation time prolonged, and there is increase in size and caliber of the peripheral venous channels. Rest in bed and medical therapy may occasion clinical improvement with disappearance

of the accumulations of fluid, and with changes of the circulation toward normal After operation in those cured, the measurements assumed normal limits and in those "improved" the measurements of the circulation approached normal In this syndrome the symptoms and signs appear to be a consequence of the defects in circulation which the constricting pericardium occasions These defects appear to be two (*1*) obstruction to entrance of blood into the chambers of the heart and (*2*) interference with contraction and emptying of the heart. These result in (*1*) decrease in cardiac output per minute and per beat and (*2*) piling up of blood on the venous side, which accounts for rise in venous pressure and slowing of the velocity of blood flow Releasing the heart and removing obstruction by resection of part of the pericardium results in return of these functions toward or to normal levels

BIBLIOGRAPHY

1 White, P D., Chronic constrictive pericarditis (Pick's disease) treated by pericardial resection. Lancet, 1935 2, 539 and 597

2. Churchill E. D Pericardial resection in chronic constrictive pericarditis. Ann. Surg 1936, 104, 516.

3 Beck, C. S , and Griswold R. A., Pericardiectomy in the treatment of the Pick syndrome. Experimental and clinical observations. Arch. Surg., 1930 21, 1064

4 Blalock quoted by Burwell (13)

5 Stewart, H. J and Heuer G J Chronic constrictive pericarditis Dynamics of the circulation and results of surgical treatment. Arch. Int. Med. (In press)

6. Grollman, A. The Cardiac Output of Man in Health and Disease. C. C. Thomas Co., Springfield 1932 p. 73

7 Grollman A., Friedman, B., Clark, G, and Harrison, T R., Studies in congestive heart failure. XXIII A critical study of methods for determining the cardiac output in patients with cardiac disease. J Clin. Invest., 1933 12, 751

8. Tarr L., Oppenheimer, B S., and Sager R. V., The circulation time in various clinical conditions determined by the use of sodium dehydrocholate Am. Heart J 1933 8, 766

9 Taylor F A., Thomas A B., and Schleiter H. G, A direct method for the estimation of venous pressure. Proc. Soc. Exper Biol and Med., 1930 27, 867

10 Levy, Robert L., The size of the heart in pneumonia. A teleoroentgenographic study with observations on the effect of digitalis therapy Arch. Int. Med., 1923 32, 359

11 Bardeen, C. R., Determination of the size of the heart by means of the x rays Am. J Anat. 1918, 23, 423

12. Burwell C. S., and Strayhorn, W D Concretio cordis. I A clinical study, with observations on the venous pressure and cardiac output. Arch Surg 1932, 24, 106

13 Burwell C. S., and Flickinger D., Obstructive pericarditis. Effect of resection of the pericardium on the circulation of a patient with concretio cordis. Arch. Int. Med., 1935 56, 250

14 Beck C. S., and Cushing, E. H., Circulatory stasis of intrapericardial origin. The clinical and surgical aspects of the Pick syndrome. J A. M A., 1934 102, 1543

15 Stewart, Harold J, and Cohn Alfred, E., Studies on the effect of the action of digitalis on the output of blood from the heart III Part I The effect on the output in normal human hearts Part II The effect on the output of hearts in heart failure with congestion in human beings J Clin. Invest. 1932, 11 917

16 Stewart, H. J Crane, N F Deitrick, J E., and Thompson, W P., Action of digitalis in compensated heart disease. Part I In the presence of regular sinus rhythm. Part II In the presence of auricular fibrillation. Arch. Int. Med. (In press)

17 Stewart, H. J., Deitrick, J E., Crane, N F., and Wheeler C. H., Action of digitalis in the uncompensated heart disease. Part I In the presence of regular sinus mechanism. Part II In the presence of auricular fibrillation. Arch. Int. Med. (In press)

18. Cohn, A. E., and Stewart, H J., Evidence that digitalis influences contraction of the heart in man. J Clin. Invest., 1924 1, 97

19 Cohn, A. E., and Stewart, H J., The relation between cardiac size and cardiac output per minute following the administration of digitalis to normal dogs. J Clin. Invest., 1928 6, 53

20 Cohn, A. E. and Stewart, H J., The relation between cardiac size and cardiac output per minute following the administration of digitalis in dogs in which the heart is enlarged. J Clin. Invest., 1928, 6 79

21 Stewart, H J., Crane, N F., and Deitrick, J E., Studies of the circulation in pernicious anemia J Clin. Invest., 1937 16, 431

22. Hitzig W M., The use of ether in measuring the circulation time from the antecubital veins to the pulmonary capillaries Am. Heart J., 1935 10, 1080

23 Gobner, R., The use of CO, inhalation and of alpha lobeline as tests of circulation time. Read before the Scientific Meeting of the New York Heart Assoc., New York Acad Med., February 1st, 1938.

RENAL EXCRETION AT LOW URINE VOLUMES AND THE MECHANISM OF OLIGURIA

By LEON C. CHESLEY

(From the Department of Biochemistry Margaret Hague Maternity Hospital, Jersey City)

(Received for publication April 5 1938)

In previous papers (1, 2) it was shown that when the urine volume output falls below about 0 35 ml per minute, the urine becomes "maximally" concentrated with respect to urea. The urea concentration of the urine is constant below this critical volume, and thus the urea clearance (U V/B) varies directly and quantitatively with the urine volume (V)

In an effort to analyze the mechanism of renal excretion at low urine volumes, the plasma clearance of endogenous creatinine, and the excretions of phosphorus, total nitrogen, and total solids have been studied in a series of oliguric subjects These findings are the basis for this report

MATERIAL AND METHODS

Series of excretion studies were carried out, using four normal non pregnant adults one normal pregnant woman at term, six patients with preeclamptic toxemia, one patient with terminal malignant nephrosclerosis and cardiac decompensation one with Bright's disease, and one unclassified cardiac patient. In the normals a thirty to sixty hour food and water fast was necessary to get the urine volume down to the desired level. In the toxemia patients, a twelve hour fast sufficed, while the patient with renal disease required no preparation Most of the urines were taken at hourly intervals, though some were for longer periods up to three hours. When the completeness of collection was controlled by washing out the bladder specimens were taken at intervals of twenty to thirty minutes In the first two normals, voided specimens were used in all other cases the urines were obtained by catheter In the later experiments the urine was first collected and then the bladder washed out twice with saline at the end of each collection. The washings were separately analyzed. The observed urine volume was corrected by adding the volume of urine calculated, from the creatinine, to be in the saline washings.

The apparent plasma creatinine was determined in a modified Folin Wu filtrate of the plasma, by the method of Folin and Wu (3) Urinary creatinine was determined by Folin's (3) method.

For the determination of urinary nitrogen 1 ml of urine was diluted to volume in a 200 ml. volumetric flask. From this were taken 2, 3, and 5 ml. samples which were digested by the Wong persulphate method (4) After cooling, they were nesslerized and read against a standard containing 0 15 or 0.25 mgm. of nitrogen. Correction was made for protein whenever present.

Inorganic phosphorus was determined in 1 2 and 5 ml. samples of the urine diluted 1 200 Youngburg's method (3) was used.

Total solids were determined indirectly because of the small amounts of urine available. Using calibrated pipettes, 10 or when necessary, 5 ml of urine were weighed to a tenth of a milligram The specific gravity was then calculated and corrected for protein (for each 10 grams of protein per liter 0.0030 was subtracted from the specific gravity) The significant figures in the specific gravity were then multiplied by Long's coefficient, 2.6, to get the approximate total solid content (3)

All data were fitted to curves derived by the method of least squares

RESULTS

As was previously reported for the excretion of urea, all substances investigated are maximally concentrated when the urine volume falls to 0.35 to 0 50 ml per minute Further decrease in volume is without effect upon the concentration of creatinine, phosphorus, total nitrogen, total solids, or total non-nitrogenous solids Like urea, their excretion at low urine volumes depends linearly and quantitatively upon the volume.

The influence of the urine volume upon the plasma clearance of endogenous creatinine is shown in Figure 1 In all cases, when the clearances are plotted against the urine volume, the best fitting curve for the data is a straight line originating close to zero on the coordinates The mean curve shown was derived by averaging the equations obtained for the different series of clearances The ratio of each line to the mean line was then calculated Each point on each line was then multiplied by the appropriate factor and plotted with reference to the mean curve. This is a simple method of averaging all observations to determine their trend and distribution The actually observed data are summarized in T I which also gives the a a describing the data.

TABLE I

Plasma clearances of endogenous creatinine at low urine volumes

Minute volume	Urine creatinine	Creatinine clearance	Plasma creatinine	Minute volume	Urine creatinine	Creatinine clearance	Plasma creatinine
ml	*mgm per 100 ml*	*ml*	*mgm per 100 ml*	*ml*	*mgm per 100 ml*	*ml*	*mgm per 100 ml*
0 317	204	43 2		0 156	312	48 7	
0 400	192	51 3		0 200	250	50 0	
0 500	195	65 0	1 5	0 225	256	57 6	
0.360	194	46 6	Preeclampsia	0 158	280	44 3	1 0
0 200	198	26 5	Y = − 1 52 + 134 V	0 187	288	53 9	Normal
0 384	267	68 3		0 202	290	58 6	Y = 1 72 + 265 V
0 250	195	32 5		0 246	280	68 9	
				0 268	250	67 0	
0 276	400	85 0		0 273	106	14 5	2 0
0 367	330	93 3		0 600	113	33 8	Nephritis
0 262	370	74 6		0 384	110	21 1	Y = − 1 + 58 V
0 300	340	78 5					
0 384	270	79 8	1 3				
0 326	290	72 7	Normal	0 083	132	9 1	1 2
0 270	360	74 6	Y = 3 5 + 248 V	0 475	144	57 0	Nephritis
0 302	340	79 4		0 329	153	42 0	Y = − 0 6 + 127 V
0 266	380	77 8		0 416	158	54 8	
0 380	300	87 7					
0 317	227	74 4		0 167	216	32 2	1 12
0 133	280	38 6	0 97	0 184	222	36 4	Preeclampsia
0 117	286	34 4	Preeclampsia	0 392	202	70 7	Y = 0 62 + 156 V
0 517	247	132 0	Y = 9 5 + 227 V	0 300	182	48 7	
0 200	282	58 2					
0 217	263	59 0					
0 738	124	83 0		0 856	177	108 3	
3 050	33	91 4		0 794	191	108 2	
1 216	78	86 5	1 10	0 855	178	109 0	1 40
1 150	89	92 5	Cardiac	0 792	200	113 2	Normal
2 000	51	93 0		0 592	272	115 0	
2 500	39	89 0		0 533	270	103 0	
0 275	248	58 2		0.318	323	73 0	
0 188	248	39 8		0 261	335	61 7	1 41
0 220	250	47 0	1 17	0 350	323	80 2	Normal
0 153	276	36 2	Preeclampsia	0 479	315	107 0	Y = 228 V
0 125	263	28 0	Bladder washed out	0 409	234	68 1	Bladder washed out
0 405	226	78 3	Y = 217 V	0 299	331	70 3	
0 420	219	78 5		0 265	314	58 8	
0 555	164	77 6					

Since the endogenous creatinine clearance has been interpreted as a measure of glomerular filtration in the human kidney (5), these results carry an important theoretical significance In the first experiments, the creatinine clearances were done with catheterized specimens, but the bladder was not washed out This leaves the possibility that the actual urine volume output may have been constant while varying quantities of the urine were left in the bladder folds To rule out this possibility, later urine collections were made quantitative by washing out the bladder at each period, as described above The results of these creatinine clearances accorded with those in the earlier cases In Figure 1, the results of the wash-out experiments are shown by the symbol X which follows the straight line as closely as do the other symbols In the pregnant patients possible retention of urine in the ureters must be considered However, the results agree for all subjects, pregnant or not, normal or not, and with bladder washed out or not The results for the minute excretion (U V) of inorganic phosphorus, total nitrogen, total solids, and total non-nitrogenous solids are shown in Figures 2, 3, and 4 The data were averaged for graphing in the same manner as described for the creatinine clearances In all cases, straight lines extrapolating back to

zero origin best describe the trend of the excretions when the excretion is plotted against the urine volume. Table II presents these data. As was the case for both urea and creatinine clearances at minimal volumes, the excretion of inorganic phosphorus, total nitrogen, and total solids depends linearly and quantitatively upon the urine volume. The same is true of the non-nitrogenous solids, obtained by subtracting total nitrogen from total solids in cases where parallel determinations were made. Here the nitrogenous solids were calculated approximately, as urea, by multiplying the total nitrogen by 2.15.

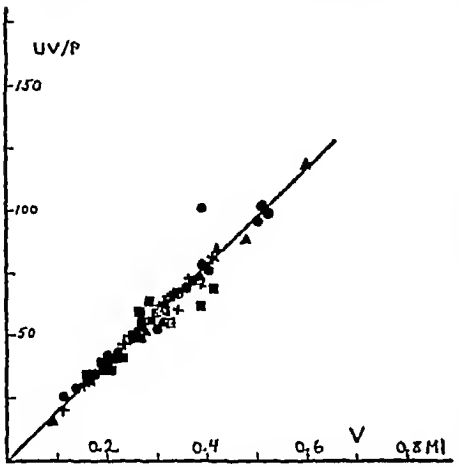


FIG. 1 PLASMA CLEARANCES OF ENDOGENOUS CREATININE AT LOW URINE VOLUMES

■ Normal subjects
▲ Preeclamptic patients
● Nephritic patients
× Data from urine collections made quantitative by washing out bladder

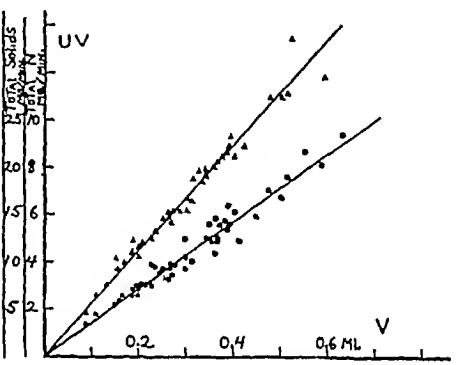


FIG. 3 TOTAL SOLID AND TOTAL NITROGEN EXCRETION AT LOW URINE VOLUMES

Total solids
● Total nitrogen.

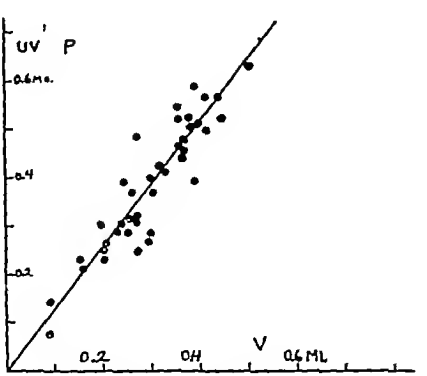


FIG. 2. INORGANIC PHOSPHORUS EXCRETION AT LOW URINE VOLUMES. ALL RESULTS FROM NORMAL, PRE ECLAMPTIC, AND NEPHRITIC SUBJECTS AVERAGED AND PLOTTED WITH REFERENCE TO MEAN CURVE, AS DESCRIBED IN TEXT

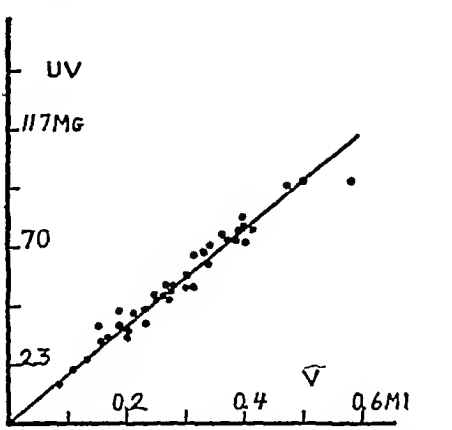


FIG 4 THE EXCRETION (U V) OF TOTAL NON NITROGENOUS SOLIDS AT LOW URINE VOLUMES. DATA OBTAINED BY SUBTRACTING (TOTAL NITROGEN × 2.15) FROM TOTAL SOLIDS

DISCUSSION

Miller and Dubos (6) have recently compared the Jaffe reaction, the new dinitrobenzoic acid colorimetric method, and a specific enzyme method in studying the plasma and whole blood creatinine They found that for plasma creatinine the values obtained by the three methods agreed very well Since the enzyme used is specific for creatinine, they concluded that the Jaffe reaction does measure fairly accurately the true creatinine level of the plasma Using this specific enzyme method, Miller and Winkler (5) found that the plasma clearance of endogenous creatinine was usually, though not always, equal to the inulin clearance. Immediately following the injection of exogenous creatinine, these workers found an abrupt rise in the creatinine clearance while the inulin clearance remained unchanged They suggested that the increased clearance of creatinine might be attributed to a tubular secretion which had been absent until the stimulus of the increased plasma level came into being This might also mean that all

TABLE II

The excretion of inorganic phosphorus, total nitrogen, and total solids at low urine volumes

Minute volume	Phosphorus	Phosphorus excretion	Total nitrogen	Nitrogen excretion	Total solids	Solid excretion	Subject and equations
ml	*mgm per ml*	*mgm per minute*	*mgm per ml*	*mgm per minute*	*mgm per ml*	*mgm per minute*	
0 317	0 814	0 258	5 08	1 61	37 4	11 82	Preeclampsia *
0 400	0 846	0 338	5 04	2 02	33 6	13 43	Phosphorus excreted
0 500	0 760	0 380	4 45	2 22	35 2	17 58	= 78 4 × Volume
0 360	0 732	0 263	3 84	1 38	36 7	13 20	Nitrogen excreted
0 200	0 755	0 151	4 00	0 80	30 7	6 14	= 47 3 × Volume
0 384	0 800	0 307	5 56	2 13	37 0	14 20	Solids excreted
0 250	0 767	0 192	4 88	1 22	37 0	9 25	= 357 × Volume
0 417	0 727	0 304	7 35	3 06			
0 350	0 917	0 321	10 08	3 53			
0 550	0 833	0 457	10 00	5 50			Preeclampsia
0 367	0 858	0.314	8 35	3 06			Phosphorus excreted
0 584	0 800	0 466	8 74	5 08			= 80 4 × Volume
0 367	0 788	0 289	9 60	3 52			Nitrogen excreted
0 633	0 816	0 516	9 32	5 90			= 89 8 × Volume
0 443	0 714	0 315	8 20	3 63			
0 355	1 023	0 364	13 62	4 84			Unclassified cardiac
0 360	1 063	0 383	16 22	5 85	Nitrogen excretion		patient
0 237	1 203	0 286	16 48	3 91	= 142 × Volume		Phosphorus excretion
0 348	1 249	0 435	13 31	4 64			= 104 8 × Volume
0 167			12 28	2 05	60 8	10 15	Preeclampsia *
0 184			11 02	2 03	59 6	10 98	Nitrogen excreted
0 392			10 84	4 25	60 8	23 86	= 111 × Volume
0 300			9 45	2 83	53 0	15 90	Solids excreted
0 234			12 72	2 98	52 7	12 30	= 572 × Volume
0 156			16 95	2 64	69 7	10 88	
0 200			16 22	3 24	55 0	11 00	
0 225			16 12	3 63	58 0	13 05	Normal *
0 158			16 40	2 59	61 2	9 68	Nitrogen excreted
0 187			18 28	3 41	69 7	13 03	= 172 × Volume
0 202			17 26	3 49	64 2	12 97	Solids excreted
0 246			17 84	4 38	62 2	15 30	= 584 × Volume
0 268			17 72	4 75	59 8	16 00	
0 276			20 40	5 63	80 0	22 10	
0 367			21 44	7 87	83 6	30 70	
0 262			20 00	5 24	86 8	22 70	
0 300			22 70	6 81	83 6	25 10	Normal *
0 384			22 42	8 62	85 8	32 95	Nitrogen excreted
0 326			24 40	7 96	91 0	29 62	= 231 × Volume
0 270			23 08	6 23	82 4	22 20	Solids excreted
0 302			27 00	8 15	82 5	24 90	= 845 × Volume
0 266			22 77	6 05	82 5	21 95	
0.380			24 50	9 31	84.5	32 10	

TABLE II—*Continued*

Minute volume	Phosphorus	Phosphorus excretion	Total nitrogen	Nitrogen excretion	Total solids	Solid excretion	Subject and equations
ml.	mgm. per ml.	mgm. per minute	mgm. per ml	mgm. per minute	mgm. per ml	mgm. per minute	
0.317			10 28	3.26	67 6	21 40	Preeclampsia *
0 200			12 44	2 48	66 4	13.29	Nitrogen excreted
0 133			12 90	1 72	63.8	8.50	= 116 × Volume
0 117			12 50	1 46	63.3	7 40	Solids excreted
0 217			11 72	2.54			= 640 × Volume
0.517			11.26	5 83	61 4	31 70	
0.083			6 68	0.55	33 8	2.80	Preeclampsia *
0 475			6 60	3 14	36 4	17.30	Nitrogen excreted
0.329			5 85	1 92	36 4	11 95	= 63.2 × Volume
0 416			6.31	2 62	33 8	14 03	Solids excreted
							= 354 × Volume
				Minute volume	Phosphorus	Phosphorus excretion	
				ml.	mgm. per ml	mgm. per minute	
0.276	2.56	0 706					
0.367	2.08	0 764		0 156	1 74	0.272	
0 262	2 06	0.540		0.200	1.36	0.272	
0.300	1 79	0.538	Normal	0.225	1 46	0.328	Normal
0.384	1 87	0 718	Phosphorus excreted	0 158	1.57	0 248	Phosphorus excreted
0.326	1 82	0.594	= 250 × Volume	0 187	1 90	0.356	= 0 116 + 93 5
0.270	1.33	0.359	− 0 19	0.202	1 47	0.297	× Volume
0.302	1.37	0 413		0.246	1.33	0.327	
0.266	1 74	0 464		0.268	1.37	0.367	
0.380	2 28	0 866					
0.533	1.274	0 682		0 657	0.390	0 256	
0 478	1 709	0 816	Normal	0 284	0 576	0 163	
0 856	0.800	0 685	Phosphorus excreted	0 241	0.588	0 142	Normal
0 792	0 952	0 753	(no equation)	0.318	0 719	0 228	
0.592	1 235	0 730		0 464	0 494	0 229	
				1 294	0 200	0.258	
0 083	0 741	0 061					
0 238	0 678	0 161	Preeclampsia				
0.292	0.372	0 108	Phosphorus excreted				
0 297	0.577	0 171	= 53.2 × Volume				
0 092	0.345	0 032					

* Simultaneous creatinine clearances (see Table I)

of the apparent plasma creatinine was not really creatinine, though the use of the specific enzyme would seem to preclude this possibility

From this we may tentatively assume that the endogenous creatinine clearance, as done in the present study, measures glomerular filtration in man, at least roughly

The quantitative dependence of the plasma creatinine clearance upon the final urine volume (or perhaps the converse), at levels below about 0.5 ml. per minute, is shown in Figure 1 On the assumption that the clearance measures glomerular filtration, it must be concluded that in oliguria the final urine volume varies directly with the filtration Several investigators have shown that at all ordinary volumes, above 0 6 ml per minute, the filtration rate is essentially constant in man The urine volume, which may vary enormously, is regulated entirely by the tubular reabsorption of water (7) Even markedly reduced glomerular filtration, on the other hand, does not usually result in a diminished output, since there is characteristically a polyuria in advanced chronic nephritis

However, the findings on excretion at low urine volumes reported here, suggest that at minimal levels the amounts of fluid filtered by the glomeruli do influence the quantity of final urine The excretion of all the substances investigated varies directly with the urine volume, in the minimal range. The simplest possible explanation for this would be that varying amounts are filtered

It is only at these minimal urine volumes that the amounts excreted do vary directly with the quantity of urine, and therefore it is only in this range of volumes that the final amount of urine bears any definite relation to the amount of fluid filtered by the glomeruli Chasis (7, 13) found that the inulin clearance showed no decrease, in man, as the urine volume fell to as low as 0 6 ml per minute This is roughly confirmed by some of the data in Table I, when the urine volume is above 0 4 to 0 5 ml per minute, the endogenous creatinine clearance is practically constant It is only below this level that the clearance shows the linear decrease to zero, as the urine volume falls

When the minute volume of urine falls to 0 35 to 0 5 ml, the concentrations of all substances studied reach their highest levels Further decrease in volume is without effect upon the concentrations, which appear to be maximal for the existing conditions Relative to the amounts of the different solids excreted, there is a constant and perhaps maximal amount of water reabsorbed at all urine volumes below the critical level Perhaps this means that the kidneys are doing a maximal amount of osmotic work, and further reabsorption of water is impossible without further reabsorption of solids

Assuming for the moment that there is a maximal tubular reabsorption of water when the urine volume falls to 0 4 ml per minute, and that the glomerular filtrate is 120 ml per minute (7), it is seen that the tubules have reabsorbed 119 6 ml of water per minute, or 99 67 per cent of the filtered volume

There is a possible alternative explanation of the findings which have been interpreted as meaning a decreasing glomerular filtration Perhaps the glomerular filtration is still constant in this range of minimal urine volumes, but the tubules reabsorb water and each of the several excretory products in exact proportion as the volume falls below the critical level The kidney must, then, deal with each substance separately, yet in quantitatively the same manner, so that the proportion of any one substance to water, and to every other substance is fixed This reabsorption must include creatinine, which has never been shown to be reabsorbed, nor even considered to be

In the lower animals, the urine volume is apparently controlled primarily by glomerular filtration In fact, the glomerulus, in the course of evolution, seems to have been developed in response to a need for excreting water (8) This control of the urine volume by glomerular filtration has been demonstrated in the frog by Marshall (9), and in the sculpin by Clarke (10) In the mammals, glomerular filtration, thanks to a coincidentally efficient tubular reabsorption of water and certain other substances, has been secondarily diverted to excretion of wastes And in most mammals, there seems to be no relation between glomerular filtration and final urine volume, at ordinary levels For the rabbit, Kaplan and Smith (11) did find that the clearances of inulin and creatinine " vary with the urine flow, in fact, these clearances fall precipitously at urine flows below 1 cc per square meter per minute " and " the present data on the excretion of inulin and creatinine point very strongly to a physiological association between glomerular function and urine flow in the rabbit " Perhaps this statement also applies for the human kidney, though only when the urine volume is very low When the tubules have reached their peak capacity in reabsorbing water, the glomeruli may revert to their primitive function in conserving water by decreasing filtration

Since the trend of creatinine clearances shown in Figure 1 extrapolates back to zero, it follows that at very small urine volumes the glomerular filtration becomes very small The question arises as to what may be the mechanism regulating the filtration Renal ischemia is a possibility reviewed by Kaplan and Smith (11) in discussing variations in glomerular filtration in the rabbit Another possibility lies in the differential contraction of the afferent and efferent glomerular vessels, which would produce changes in glomerular pressure Changes in glomerular pressure probably do occur on this basis, the literature is reviewed by Winton (12) Whatever the mechanism, there is the further question as to the nature of the stimulus eliciting the changes in glomerular filtration

SUMMARY AND CONCLUSIONS

When the urine volume falls below a critical limit of about 0 35 to 0 5 ml per minute (21 to 30 ml per hour, 504 to 720 ml per 24 hours) urea, creatinine, inorganic phosphorus, total ni-

trogen, total non nitrogenous solids, and total solids become maximally concentrated Further reduction in urine volume does not increase the concentration.

The plasma clearance of endogenous creatinine, as well as the excretion of the other substances studied, shows a quantitative linear dependence upon the urine volume, in the minimal range. It is therefore suggested that these urine volumes vary with glomerular filtration This implies that a constant and perhaps maximal proportion of the filtered water is reabsorbed by the tubules

I am indebted to Dr S A. Cosgrove for his interest in this work, and for the use of his private as well as ward patients

BIBLIOGRAPHY

1 Chesley, L. C., The validity of the calculation of standard urea clearances from low urine volumes. J Clin. Invest., 1937, 16 653
2. Chesley, L. C., Urea excretion at low urine volumes The calculation of "minimal" urea clearances J Clin. Invest., 1938 17, 119
3 Hawk, P B., and Bergeim, O., Practical Physiological Chemistry Blakiston s Sons, Philadelphia, 1931 10th ed., pp 421, 813 835, 876
4 Peters J P., and Van Slyke D D Quantitative Clinical Chemistry Vol. II. Methods Williams and Wilkins Co., Baltimore, 1932, p. 527
5 Miller B F, and Winkler, A W., The renal excretion of endogenous creatinine in man. Comparison with exogenous creatinine and inulin J Clin. Invest., 1938, 17, 31
6 Miller B F., and Dubos, R., Studies on the presence of creatinine in human blood. J Biol. Chem., 1937 121, 447
7 Smith H. W., The Physiology of the Kidney Oxford University Press, New York, 1937, pp 64 to 68
8 Marshall E. K., Jr., The comparative physiology of the kidney in relation to theories of renal secretion Physiol. Rev., 1934 14, 133
9 Marshall, E. K. Jr., The secretion of urea in the frog J Cell. and Comp Physiol., 1932, 2, 349
10 Clarke, R. W., The xylose clearance of Myoxocephalus octodecimspinosus under normal and diuretic conditions J Cell. and Comp Physiol., 1934, 5, 73
11 Kaplan B I., and Smith, H W., Excretion of inulin creatinine, xylose and urea in the normal rabbit. Am. J Physiol., 1935 113, 354
12. Winton, F R., Physical factors involved in the activities of the mammalian kidney Physiol Rev., 1937, 17, 408.
13 Chasis, H., Unpublished data.

THE VALUE OF THE ACID TEST MEAL A STUDY OF NORMAL PERSONS AND OF PERSONS WITH DUODENAL ULCER

By C. STUART WELCH AND MANDRED W COMFORT

(From The Mayo Foundation and the Division of Medicine The Mayo Clinic Rochester Minn.)

(Received for publication April 5, 1938)

Much evidence has been accumulated to show that the separate secretions of the gastro-intestinal tract have a definite and invariable composition under normal conditions (1) These secretions are isotonic with blood serum, each has its own acid-base pattern In the stomach two main fluids arise from different groups of cells The first is a secretion of isotonic hydrochloric acid from the chief cells of the body and fundus of the stomach, the second is a slightly alkaline fluid which probably is secreted for the most part by the antral mucosa Hydrochloric acid is secreted by the stomach in the concentration of approximately 600 mgm of chloride for each 100 cc. (2, 3, 4), although its concentration in mixed gastric contents may show wide variations in health and disease. Five other non-acid secretions, in addition to that elaborated by the antral mucosa, enter the stomach and thus affect the concentration of acid in the final mixture. These fluids are saliva, pancreatic juice, bile, mucus, and intestinal juice. Pancreatic juice, bile, and intestinal juice are likewise isotonic with blood serum The alkaline fluid secreted by the stomach and the fluids which enter the stomach from the duodenum have a tendency to reduce the concentration of isotonic hydrochloric acid in the gastric contents Thus is accomplished by dilution more than it is by neutralization The importance of the factor of neutralization will depend largely on the alkalinity as well as on the quantity of the duodenal fluid regurgitated into the stomach. On the other hand the secretion of more isotonic hydrochloric acid by the fundic cells will tend to conteract the effect of the addition of alkaline fluids and to maintain the normal concentration of the hydrochloric acid. The concentration of hydrochloric acid in the stomach at any one time will depend on the volume and concentration of acid and alkaline material that are mixed together

The usual gastric test meal furnishes information concerning the concentration of free hydrochloric acid in the gastric contents after dilution and neutralization but gives little information concerning the relative amounts of acids and alkaline fluids that enter the stomach during the test period A method of study that has been evolved by Wilhelmj, Neigus, and Hill (3) promises to furnish some information about this point For this reason, a clinical appraisal of the method, after defining its limitations, seemed appropriate. Specifically, we were interested in ascertaining the clinical value of the method, in finding to what extent dilution, neutralization, and secretion of chloride as hydrochloric acid during the test period could be measured, in observing the influence of regurgitation of duodenal fluids on the concentration of hydrochloric acid in the test meal, and finally in contrasting the effectiveness of dilution, neutralization, and secretion of hydrochloric acid in reducing and maintaining the concentration of hydrochloric acid introduced into the intact stomach of normal persons and of patients who had duodenal ulcer Twenty five normal persons and thirty persons who had duodenal ulcer kindly consented to submit to this test in order that we might ascertain the clinical value of the method

METHOD

The method (3) is fundamentally a simple one. Three hundred cubic centimeters of approximately 0 1 normal solution of hydrochloric acid that contains phenol red in the amount recommended by Wilhelmj, Neigus, and Hill (3) is placed in the stomach and allowed to remain there thirty minutes after which time the contents of the stomach are aspirated as completely as possible. The test is repeated two or three times at one sitting Preliminary preparation includes a fast for fifteen hours and lavage of the stomach with the test solution before the beginning of each test, in order to avoid as much as possible the effect of immediate dilution. The gastric contents are removed thirty minutes after the instillation of the test solution and are examined for the concentration of total chloride, the concentration of acid chloride (chloride as hydrochloric acid) and the concentration of phenol red. The method of Van Slyke (5) is used for estimation of total chloride. The con

centration of acid chloride is estimated from the concentration of free hydrochloric acid as determined by titration with a 0 1 normal solution of sodium hydroxide by using dimethylaminoazobenzene as an indicator The concentrations are expressed in terms of milligrams of chloride per 100 cc of gastric contents The concentration of phenol red in the gastric contents is determined by the method outlined by Wilhelmj, Neigus, and Hill (3), and is expressed as percentage of the concentration of phenol red in the original test solution For example, if the concentration of phenol red in the solution introduced into the stomach was 1 per cent, and the concentration of phenol red in the aspirated gastric contents was 0 5 per cent, the concentration of phenol red in this aspirated gastric contents would be expressed as 50 per cent As will appear in the next paragraph, another subtraction remains to be made to secure the final result.

Calculation The amount of dilution, that is, the number of cubic centimeters of various secretions which entered the stomach in the test periods per 100 cc. of gastric contents removed, can be determined by subtracting the concentration of phenol red in the aspirated gastric contents (expressed as percentage of concentration in the original test solution) from 100 In the example, then, 50 would be subtracted from 100 and the final result is 50, which represents the number of cubic centimeters of dilution per 100 cc. of aspirated contents

The diluting effect of this volume of fluid on the concentration of acid in the original test solution is estimated by multiplying the concentration of acid chloride in the original test solution by the percentage of phenol red in the recovered gastric contents This constitutes the correction for dilution The amount of acid chloride of the test solution that has been neutralized (neutralized chloride) is determined by subtracting the concentration of acid chloride in the gastric contents from the concentration of acid chloride in the original test solution corrected for dilution The value for the neutralized chloride may be determined only when the concentration of acid chloride, as obtained by analysis, is less than the concentration of acid chloride in the test solution corrected for dilution The concentration of acid chloride that is effective in increasing the concentration of acid chloride in the test solution after correction for dilution is determined by subtracting the concentration of acid chloride of the original test solution, which has been corrected for dilution, from the concentration of acid chloride in the gastric contents This value for the extra acid chloride may be determined only when the concentration of acid chloride in the gastric contents is greater than the concentration of acid chloride in the test solution which has been corrected for dilution If it is found that the concentration of acid in the gastric contents is less than could be accounted for by physical dilution, as determined by the change in concentration of phenol red, it shows that the various secretions which entered the stomach and mixed with the test meal contained alkali in excess of acid On the other hand, if the concentration of acid in the gastric contents exceeds the amount expected after correction for dilution, it shows, conversely, that the secretions added to the test meal contained hydrochloric acid in excess of neutralizing material Thus, the analyses and calculations which are employed in determining the type of fluid which enters the stomach and mixes with the acid test meal depend in a large measure on the estimation of the dilution which the test meal has undergone. These calculations will be more fully considered when the clinical application of the method is reached

THE EXTENT TO WHICH THE EFFECT OF ENTRANCE OF ALKALINE FLUID AND ACID FLUID INTO THE STOMACH CAN BE MEASURED

The results naturally divided the tests into two groups first, those in which more alkaline secretions than acid secretions entered the stomach, and second, those in which more acid secretions than alkaline secretions entered the stomach Typical experiments of each type will be described

There is ample evidence that secretions which enter a stomach that is capable of secreting hydrochloric acid and contains an acid test meal are composed of both acid and alkali The observations of Pavlov (6), Ivy and Whitlow (7), MacLean, Griffiths, and Williams (8), MacLean and Griffiths (9, 10), Apperly (11), and Apperly and Norris (12) have demonstrated that acid secretion is inhibited when N/10 solution of hydrochloric acid is placed in the stomach, but the observations of Apperly and Norris (12), and Wilhelmj, O'Brien, and Hill (13) clearly demonstrate that the acid test meal does not always or completely inhibit the secretion of acid by the stomach Apperly and Norris found that in a small percentage of cases hydrochloric acid was secreted after the introduction of 0 08 normal to 0 128 normal solutions of hydrochloric acid, and Wilhelmj, O'Brien, and Hill demonstrated that the secretion of hydrochloric acid by the gastric mucosa of dogs was not completely inhibited by a 0 1 normal solution of hydrochloric acid In previous clinical studies in which the acidity of the gastric contents was determined by using the acid test meal (14, 15, 16), the contrary has been assumed, namely, that the introduction of an approximately 0 1 normal solution of hydrochloric acid completely inhibited the secretion of hydrochloric acid by the stomach Similarly, evidence may be cited that alkaline fluid usually, if not al-

ways, enters the stomach when it contains an acid test meal First, bile pigments frequently are present in the gastric contents, but the absence of bile pigments does not necessarily indicate an absence of duodenal regurgitation Spencer, Meyer, Rehfuss, and Hawk (17) demonstrated that a tryptic enzyme is almost constantly present in the digestive contents of the normal stomach and concluded that the trypsin was regurgitated from the duodenum Medes and Wright (18) have likewise demonstrated that duodenal regurgitation without bile pigment is a frequent occurrence. Second, Wilhelmj, Henrich, Neigus, and Hill (4), by using the acid test meal, showed that antral secretion into the stomach of dogs is continuous and that the volume secreted in thirty minutes varies from 2 to 15 cc and is apparently independent of the amount of acid secretion The results of our experiments demonstrate that secretions which enter an intact stomach that is capable of secreting hydrochloric acid, during the thirty minute period, are composed usually, if not always, of both acid and alkali. Obviously, this is not true if the stomach is unable to secrete hydrochloric acid

Changes in concentration caused by the entrance of more alkaline secretions than acid secretions An acid solution which has a concentration of 358 mgm. of acid chloride per 100 cc. was introduced into the stomach of Subject 1 at the beginning of the first test period (Table I) After thirty minutes, the solution contained phenol red in a concentration of only 73 per cent of the original concentration In other words, 27 cc. of fluid per 100 cc. of contents had entered the stomach during the test period If this fluid had been water the concentration of acid chloride in the contents removed at the end of the test period would have been 261 mgm per 100 cc. and the concentration of acid chloride in the original acid solution would have been reduced 97 mgm per 100 cc. Actually, however, the concentration of acid chloride in the original acid solution was reduced not 97 mgm, but 124 mgm per 100 cc., and the concentration of acid chloride in the gastric content was not 261 but 234 mgm per 100 cc The reduction in concentration was greater than that expected from dilution alone, and a further reduction of acid concentration resulted from neutralization Three-fourths (97 mgm) of the reduction in concentration of acid chloride introduced into the stomach as a test solution was accomplished by the factor of dilution and a fourth (27 mgm) was accomplished by neutralization The proportion of the reduction due to dilution and to neutralization is similar to that shown by Wil

TABLE I

Results of tests performed on three normal persons

	Subject 1		Subject 2		Subject 3		
	Test 1	Test 2	Test 1	Test 2	Before administration of secretin		After administration of secretin
					Test 1	Test 2	Test 3
Mgm of acid chloride in 100 cc. of test solution	358	358	342	342	364	364	364
Concentration of phenol red in gastric contents expressed as percentage of original concentration in the test solution	73	66	49	40	68	63	52
Cc of dilution per 100 cc. of gastric contents	27	34	51	60	32	37	48
Estimated concentration of acid chloride in test solution after dilution, *mgm per 100 cc* (equals correction for dilution)	261	236	168	137	248	229	189
Possible reduction in concentration of acid chloride in test solution as a result of dilution *mgm per 100 cc.*	97	122	174	205	116	135	175
Actual concentration of acid chloride in gastric contents at end of test period *mgm per 100 cc*	234	197	326	292	246	239	88
Actual reduction in concentration of acid chloride in test solution, *mgm per 100 cc*	124	161	16	50	118	125	276
Amount of acid chloride of test solution neutralized during the test period *mgm per 100 cc*	27	39			2		101
Amount of acid chloride added during the test period *mgm per 100 cc.*			158	155		10	

Dr. Robert Reilly Library
U S Medical College

helmj, Henrich, and Hill (19) when mixed duodenal secretions were added to acid gastric contents, and suggests that very little acid entered the stomach during the test period The results obtained in the second test which was carried out on Subject 1 during the thirty-minute period following the conclusion of the first thirty minutes (Table I) are practically identical with those obtained in the first test

It should be clearly understood that the 27 mgm of acid chloride neutralized were milligrams of acid chloride per 100 cc of the original acid solution and that these 27 mgm per 100 cc of the original acid test solution probably do not represent all the acid chloride neutralized by the alkaline fluid that entered the stomach during the test period It is most important to note that any acid chloride secreted during the thirty-minute period was likewise neutralized as well as the 27 mgm of acid chloride per 100 cc of the test solution The method does not permit measurement of this fraction of neutralization or of the total neutralization that occurs during the test period On the other hand, the 124 mgm reduction in the concentration of acid chloride in each 100 cc. of the test fluid is the net result of the reduction of concentration by dilution and neutralization and of the increase in concentration caused by the entrance of pure acid gastric juice that contains acid chloride in a concentration greater than the concentration in the test solution (approximately 600 mgm per 100 cc) The reduction in concentration in this case is probably not a true measure of all the dilution and neutralization which the alkaline fluid that enters the stomach is capable of producing but is a measure of dilution and neutralization after neutralization of the acid chloride secreted by the stomach

The results of Tests 1 and 2 on Subject 1 (Table I) are typical of the results of tests performed when fluid that enters the stomach contains little acid and is predominantly alkaline The results of Test 1 on Subject 3, on the contrary, are typical of the results obtained when only slightly more alkaline than acid material is present in the fluid that enters the stomach If the results of this test are analyzed in a manner similar to that in which Tests 1 and 2 on Subject 1 were analyzed, it will be found that the same conclusions may be drawn In other words, so long as the alkaline elements predominate over the acid elements in the combined fluids that enter the stomach during the test period, whether the predominance be great or small, the method permits measurement of reduction in concentration of acid chloride in the test fluid by dilution and measurement of reduction in concentration caused by neutralization The reduction in concentration caused by dilution is always 100 per cent while that caused by neutralization varies from a negligible amount to as much as a fourth of the total reduction The method does not permit, under these circumstances, a measurement of the acid secreted and neutralized or an estimation of the total neutralization that occurs during the test period

Changes in concentration caused by the entrance of more acid fluid than alkaline fluid A solution containing acid chloride in a concentration of 342 mgm per 100 cc was introduced into the stomach of Subject 2 at the beginning of the first test period (Table I) The percentage of the original concentration of phenol red in the gastric contents recovered after thirty minutes was 49 In other words, the volume of fluid which entered the stomach, per 100 cc of gastric contents, during this period was 51 cc If the fluid which entered the stomach had been water, the concentration of chloride in the gastric contents recovered after thirty minutes would have been 168 mgm per 100 cc and the original concentration of acid chloride would have been reduced by 174 mgm per 100 cc (51 per cent) Actually, the concentration of acid chloride in the gastric contents recovered at the end of thirty minutes was much greater (326 mgm per 100 cc) and the actual reduction in concentration was only 16 mgm The results obtained in Test 2, which was carried out on Subject 2 during the second thirty-minute period, are practically identical with those of Test 1

The reduction in concentration of acid chloride in the test solution was only 9 2 per cent of the reduction that would have been accomplished had the fluid which entered the stomach been water, moreover, no neutralization of the test solution was measurable Instead, at least 158 mgm of acid chloride per 100 cc of test solution had been added during the test period Undoubtedly, some

neutralization occurred but the amount of acid chloride neutralized is unknown.

Approximately 26 cc. of pure fundic acid secretion per 100 cc. of gastric contents is sufficient to add 158 mgm of acid chloride per 100 cc of gastric contents The 51 cc. of fluid, per 100 cc. of gastric contents, that entered the stomach contained not only 26 cc. of pure acid chloride secretion and added not only 158 mgm per 100 cc. of acid chloride but also added additional acid chloride that was sufficient to neutralize all the alkali in the remaining 25 cc. of combined fluid that entered the stomach. The 158 mgm. of acid chloride does not represent the total amount of acid chloride added during the test period but represents only that part of the added acid chloride calculable after neutralization of the alkali in the combined fluids that entered the stomach The amount of acid chloride neutralized is not measurable by the method

Tests 1 and 2 on Subject 2 are typical of tests in which more acid material than alkaline material is present in the fluid that enters the stomach during the test period The same is true of Test 2 on Subject 3 In Tests 1 and 2 on Subject 2 the acid material is greatly in excess of the alkaline material, whereas in Test 2 on Subject 3 the acid material is only slightly in excess of the alkaline material If the results of Test 2 on Subject 3 are analyzed as were results of Test 1 on Subject 2, the same conclusions may be reached In other words, whenever the acid material predominates over the alkaline substance in the combined duodenal and gastric fluids that enter the stomach, the method measures reduction in concentration of acid chloride in the test fluid, reduction in the concentration caused by dilution, and the predominance of acid chloride over alkali in the combined solutions that enter the stomach. The reduction in concentration of the test solutions is less than that expected from the addition of water alone and may be accounted for by the factor of dilution Neutralization does not reduce the concentration of acid chloride in the test solution The method permits measurement of only part of the acid chloride secreted during the test period, it does not permit measurement of the alkali that enters the stomach or measurement of the extent of neutralization of acid that enters the stomach.

COMMENT

Regardless of the type of fluid that enters the stomach the method permits measurement of the excess of alkali or acid in the combined fluids that enter the stomach When the fluid is predominantly alkaline, the excess of alkali over acid in the combined fluids that enter the stomach is measured When the fluid is predominantly acid, the excess of acid over alkali is measured

When the combined secretion that enters the stomach contains more alkali than acid the combined secretions dilute the test solution as much as water, dilution is 100 per cent effective, the combined secretions neutralize some of the test solution, and the reduction in concentration of the acid chloride of the test solution is caused both by dilution and neutralization The reduction in concentration of acid chloride of the test solution is the measure of the effect of dilution and neutralization on the test fluid, after neutralization of all acid chloride secreted by the stomach.

When the combined secretions that enter the stomach contain more acid than alkali, the combined secretions dilute the test solution less than water That is, dilution is less than 100 per cent effective The combined fluids do not neutralize any of the acid chloride in the test solution. The combined secretions add acid chloride to the test solution, and thus tend to maintain the original concentration of acid chloride that is introduced into the stomach The reduction in concentration of test solution may be accounted for entirely by the factor of dilution.

The reduction in the concentration of test solution is the net result of dilution and neutralization on the one hand and the addition of acid chloride on the other Dilution is 100 per cent effective so long as the alkaline substances in the test fluid are greater than the acid substances Dilution is less effective as the proportion of acid fluid in the total fluid that enters the stomach becomes greater

At the beginning of this analysis of the method we cited evidence that the introduction of acid chloride in a concentration of approximately normal did not always inhibit completely secretion of acid by the stomach during a test period. In this connection it should be noted that the acid chloride secreted by

measurable in 40 per cent of the tests on normal persons and in 84 per cent of the tests on persons who had duodenal ulcer (Table II) Certainly,

TABLE II

Results obtained when acid test meals were administered to normal persons and to patients who had duodenal ulcer

	Normal persons (30 tests)	Persons who had duodenal ulcer (38 tests)
Cubic centimeter of dilution (average) per 100 cc of gastric contents	23	33
Average reduction in concentration of acid chloride, *mgm per 100 cc*	76	67
Percentage of cases in which there was a demonstrable neutralization of acid chloride in test solution	60	16
Percentage of cases in which there was an increase in concentration of acid chloride, after correction for dilution	40	84

in about 65 per cent of our experiments acid chloride was secreted by the stomach during the test period in spite of the introduction of 0 1 normal solution of hydrochloric acid The inhibition of acid secretion by the introduction of a solution of 0 1 normal hydrochloric acid into the stomach was much less in the cases of duodenal ulcers than it was among normal persons

The effect of increasing the amount of alkaline fluid that enters the stomach during the test period on dilution and neutralization We found that regurgitation of the duodenal contents often follows the intravenous injection of secretin or decholin In this way we were able to produce regurgitation and study the effect of regurgitation of the test meal in the intact human stomach

In Tests 1 and 2 on Subject 3 (Table I), 32 and 37 cc of combined duodenal fluid and gastric fluid entered the stomach during the test meal and reduced the concentration of the acid chloride of the test solution 118 and 125 mgm , respectively In Test 1, 2 mgm of acid chloride of the test solution was neutralized, while in Test 2, 10 mgm of extra acid chloride per 100 cc of gastric contents was added In other words, the factors that regulate acidity of the gastric contents acted similarly on the concentration of the acid test meal in the two tests Dilution was about 100 per cent effective, neutralization was negligible, and the acid material and alkaline material in the fluids that entered the stomach were approximately equal At the beginning of the third consecutive thirty-minute test period, 30 mgm of purified secretin was administered intravenously During the third test period, an increased amount of fluid (48 cc instead of about 35 cc.) entered the stomach The reduction in concentration of acid chloride in the test fluid increased from about 120 mgm to 276 mgm per 100 cc of test solution After the administration of secretin and after regurgitation had occurred, dilution reduced the concentration of acid chloride 175 mgm instead of about 120 mgm per 100 cc of test fluid and was 100 per cent effective just as it was before the administration of secretin and before regurgitation, neutralization became definitely effective and reduced the concentration of the acid chloride in the test solution about 101 mgm per 100 cc, and the alkaline material markedly predominated over the acid material in the fluid that entered the stomach It should be noted especially that the total reduction in the concentration of acid chloride of the test solution was affected 63 per cent instead of approximately 100 per cent by dilution after regurgitation was induced, and 37 per cent instead of 0 per cent by neutralization after regurgitation was induced

In this experiment the amounts of acid material and alkaline material in the combined fluids that entered the stomach during the preliminary test periods were approximately equal, following the administration of secretin, additional alkaline fluid entered the stomach, the amount of acid became definitely less than the alkaline material in the combined fluids that entered the stomach, the concentration of acid chloride in the test solution was reduced beyond the reduction seen during the preliminary periods, the reduction caused by dilution was materially increased, and neutralization, which either was not measurable or was measurable to only a slight extent during preliminary tests, became measurable and accounted for approximately 37 per cent of the total reduction in concentration of the acid chloride in the test solution In other experiments in which the amount of acid was routinely greater than the alkali in the combined fluid that entered the stomach during the

preliminary test periods, the regurgitation of alkaline fluid was sufficient to reverse the ratio of acid to alkaline material in the combined fluids, to render dilution 100 per cent effective, and to increase neutralization, which was not measurable in the preliminary test periods, to the point where it was measurable and accounted for a considerable portion of the total reduction in the concentration of acid chloride in the test solution Such experiments clearly show the effectiveness of duodenal fluid in reducing the concentration of hydrochloric acid in the gastric contents and demonstrate that the reduction is accomplished chiefly by dilution and to a lesser extent by neutralization, as it is in dogs (19)

A comparison of factors of dilution and neutralization with the factor of acid secretion in normal persons and in cases of duodenal ulcer The acid test meal was administered on thirty occasions to normal subjects and on thirty eight occasions to patients who had duodenal ulcer The data in Table II show interesting but contrasting tendencies in the action of the factors that regulate the acidity of the gastric contents in the two groups An average of 33 cc. of fluid per 100 cc. of gastric content entered the stomachs of the patients who had duodenal ulcer and an average of 23 cc. of fluid entered the stomachs of normal persons, that is, the average amount of fluid that entered the stomachs of persons who had duodenal ulcer was greater by 10 cc. than the average amount of fluid that entered the stomachs of normal persons The average reduction in the concentration of acid chloride in the test solution was 67 mgm. per 100 cc. for the group of persons who had duodenal ulcer and 76 mgm per 100 cc. for the group of normal persons, that is, the average reductions in concentration were less by 9 mgm per 100 cc., or 12 per cent, for the group of patients who had duodenal ulcer than it was for the group of normal persons

This combination of findings, that is, the fact that more fluid entered the stomachs and less reduction occurred in the concentration of acid chloride in the test solution in cases of duodenal ulcer than occurred among normal persons, means that, on the average, more pure acid secretion entered the stomachs of the patients who had duodenal ulcer than entered the stomachs of normal persons This is in conformity with the well-recognized fact that patients who have duodenal ulcer on an average secrete a larger volume of highly acid fluid following stimulation with histamine than do normal persons

Neutralization of the acid chloride of the test solution was measurable in 60 per cent of the group of normal persons, but in only 16 per cent of the group of persons who had duodenal ulcer Again, while acid chloride secreted by the stomach (extra acid chloride) was measurable in only 40 per cent of the group of normal persons it was measurable in 84 per cent of the group of persons who had duodenal ulcer We have just pointed out that neutralization of acid chloride of the test solution is measurable by this method only when alkali exceeds acid, and acid chloride secreted by the stomach is measurable only when acid chloride exceeds alkali in the fluids that enter the stomach during the test period In other words, while the alkali exceeds the acid chloride in the fluid that enters the stomach during the test period in about four times as many normal persons as it does in persons who have duodenal ulcer, acid chloride exceeds alkali in about five times as many persons who have duodenal ulcer as it does among normal persons Since dilution is 100 per cent effective so long as alkaline material exceeds acid material in the fluid that enters the stomach, it will be seen that dilution was 100 per cent effective among 60 per cent of normal persons, but in only 16 per cent of persons who had duodenal ulcer Dilution is usually much more effective among normal persons than it is among persons who have duodenal ulcer

The greater average reduction that occurred in the concentration of acid chloride in the test solution in the case of normal persons results from the greater frequency with which alkali exceeds acid in the fluid that enters the stomach and consequently from the greater effectiveness of neutralization and dilution in the case of normal persons Elman (15), on the basis of data obtained while using the acid test meal on dogs, and Levy (16), on the basis of data obtained while using the acid test meal on man, concluded that the smaller reductions of concentration in acid chloride in the test solution in cases of duodenal ulcer was attributable to an actual deficiency in neutralization In order to reach such conclusions from the experimental data, these authors assumed

PLACENTAL INTERCHANGE II COMPARISON OF THE TOTAL BASE CONCENTRATION OF THE FETAL AND MATERNAL BLOOD AT PARTURITION

By H E. THOMPSON, JR., AND W T POMMERENKE

(Department of Obstetrics and Gynecology University of Rochester, School of Medicine and Dentistry Rochester, New York)

(Received for publication April 11 1938)

Although the literature on the permeability of the placenta to many substances either of a metabolic or foreign origin is extensive, there is a paucity of data on comparisons of maternal and infant total base. In 1920 Stander and Tyler (1) reported identical values for the ash of maternal and fetal plasma, while Lévy-Solal, Dalsace, and Gutman (2) in 1934 reported the maternal and fetal plasma ash to be 7 97 and 9.25 grams per liter respectively Eastman, Geiling, and De Lawder (3) found the total serum base of fetal and maternal serum to be low and stated that the normal adult total base was about 154 0 m eq, while that of fetal blood was about 148 m eq

In the present study, the total base was determined simultaneously on samples of maternal venous, umbilical arterial, and umbilical venous plasma from blood obtained at the time of delivery

METHODS

The subjects of this study were 30 parturient women and their respective normal appearing newborn children, No discriminations as to age, race, previous history of the mother or the weight or sex of the infant were made. However, infants born in a debilitated condition and their respective mothers were not among those on whom data is reported here. As has been noted in a previous paper (4) values for maternal blood are regarded as approximating the fasting level because little food is ordinarily consumed during labor

Collection of blood At delivery of the infant, the umbilical cord was cut and blood collected from the umbilical arteries and the umbilical vein. The blood was received into a centrifuge tube to which 10 mgm. of heparin were added to prevent clotting In only a very few instances was there clot formation. Shortly after collection of fetal blood, blood was withdrawn without stasis from a maternal arm vein and treated to prevent clotting as described above. All blood collections were generally completed within about 5 minutes. Centrifugalization for 20 minutes at moderate speed was then carried out, and the hematocrit determined. All volumetric apparatus employed in the total base analysis was calibrated by the weight method before beginning this study In order to measure accurately 0.2 cc. of plasma 1 cc. of plasma was diluted to 10 cc., and 2 cc. aliquots of this solution were used in each analysis Each determination was made in duplicate and 2 blanks were run with each dialysis, 8 cells being run at a time.

Determination of total base The rapid and efficient electrodialysis method for total base determination developed not long ago by Keys (5) was employed with gratifying results The mercury employed was purified by vacuum distillation Checks indicated that stock distilled water was as satisfactory as that obtained by distillation with phosphoric acid. Using as control a salt solution containing 130.5 m.eq of sodium and 4.5 m.eq of potassium, the mean of 7 consecutive determinations of total base was found to be 134 6 ± 0 7 m.eq The deviation of the mean from the expected value was —0.3 per cent. Since plasma solutions are far more complex in character than a simple salt solution, several determinations were made in triplicate on plasma solutions in order to determine how precise the method was under the conditions of these experiments The results are indicated in Table I.

The data show that the difference between the lowest and highest values for 3 determinations on the same plasma solution varied from 0 1 to 3 1 m.eq. with an average value of 1.8 m.eq Each determination is the mean of two analyses the agreement of which is typical, excepting for values in determination E-1 The precision in this instance is less than for any value reported in Table II

TABLE I

Precision in analyses of plasma solutions for total base

Subject	Determination 1		Determination 2		Determination 3		Difference between highest and lowest means
		Mean		Mean		Mean	
A	154 6		153 6		153 7		
	155 0	154.8	152.9	153.3	153 7	153 7	1.5
B	151 6		151 4				
	151 9	151.8	152 0	151 7	153 4	153 4	1 7
C	151 9		150 4		154.2		
	153.2	152 6	151 9	151 2	154 4	154.3	3 1
D	152.9		149 9		150 4		
	151 6	152.3	149 9	149 9	150 4	150 4	2 4
E	151 4		148.5		149 0		
	145.5	148 4	148 0	148.3	147.8	148 4	0 1
Average		152 0		150 9		152 0	1 8

± 3 5 m eq , infant venous plasma 146 8 ± 3 2 m eq The mean difference between maternal and infant arterial plasma was found to be 2.2 m eq Differences between the mean infant arterial and mean venous values are less than the experimental error Correlations of values obtained with previous history, age, number of previous children, or sex of infant were not apparent

In 13 of these cases, the plasma total base of the mother was followed after delivery, and in every instance there was a definite increase in its concentration during the puerperium

BIBLIOGRAPHY

1 Stander, H J , and Tyler, Margaret, The moisture and ash of maternal and fetal blood Surg , Gynec. and Obst., 1920, 31, 276

2 Lévy-Solal, E , Dalsace, J , and Gutman, C., Recherches biochemiques sur le role du placenta. Compt. rend. Soc de Biol , 1934, 115, 269

3 Eastman, N J , Geiling, E M K., and DeLawder, A M , Fetal blood studies IV The oxygen and carbon dioxide dissociation curves of fetal blood. Bull. Johns Hopkins Hosp , 1933, 53, 246

4 Pommerenke, W T , Placental interchange. I On the concentration of certain nitrogenous substances in the blood, before and after passing through the placenta. J Clin. Invest., 1936, 15, 485

5 Keys, A , The determination of total base in blood and other biological fluids by the electrodialysis method of Adair and Keys J Biol Chem , 1936, 114, 449

6 Bock, A , Über das Verhalten des Ammoniaks im Blute von Schwangeren, über seine Ursprungssubstanz und seine Rolle bei der Neutralitätsregulierung Arch. f Gynäk., 1930, 140, 1

7 Marx, Helmut, Der Wasserhaushalt des gesunden und kranken Menschen. J Springer, Berlin, 1935, p 31

8 Oberst, F W , and Plass, E. D , Water concentration of the blood during pregnancy, labor, and the puerperium Am J Obst. and Gynec., 1936, 31, 61

9 Dieckmann, W J , and Wegner, C R , Studies of the blood in normal pregnancy V Conductivity, total base, chloride, and acid-base equilibrium. Arch Int. Med., 1934, 53, 527

AN ATTEMPT TO INCREASE RESISTANCE TO PERTUSSIS IN NEWBORN INFANTS BY IMMUNIZING THEIR MOTHERS DURING PREGNANCY[1]

By JOHN A LICHTY, JR., BETTY SLAVIN[2] AND WILLIAM L. BRADFORD

(From the Departments of Pediatrics and Bacteriology, University of Rochester School of Medicine and Dentistry and the Strong Memorial and Rochester Municipal Hospitals Rochester New York)

(Received for publication April 22, 1938)

It is rather generally accepted that many infants in the first half-year of life are not susceptible to some of the common infectious diseases, such as diphtheria, poliomyelitis, scarlet fever, or measles (1) This resistance is particularly striking in the infant whose mother is known to have had the disease in question. Numerous investigators have shown by experimental work, in human beings as well as in animals, that this immunity coincides with the presence of specific antibodies which have been passively transferred from the mother to the offspring No attempt is made to give a complete review of this subject for it is surveyed in the publications of Ratner *et al* (2) and Aycock and Kramer (3) In man, the transfer of antibody probably occurs chiefly by way of the placenta

The incidence of pertussis is high in newborn infants Pfaundler and Schlossmann (4) give mortality rates between 26 and 55 per cent during the first year of life, and Griffith and Mitchell (5) state "the danger being greater the younger the child"

Within recent years numerous attempts have been made to immunize children actively against this disease. It is not within the scope of this paper to discuss the efficacy of this procedure or the various techniques employed, but it may be stated safely that most advocates of active immunization recommend that it be carried out during the second half-year of life. This period coincides with that used for other types of active immunization, because it is believed that very young infants do not respond to antigenic stimuli in any degree comparable to that of older individuals In the case of pertussis, unfortunately, this plan of active immunization leaves the child unprotected during that period of life when the mortality is highest.

In view of the above facts it seemed worth while to immunize women during pregnancy with a vaccine prepared with *Hemophilus pertussis*, in the hope that antibodies so produced might be transferred to the fetus The experiment was suggested further by the reports which follow

Burckhardt (6) found that infants born of mothers who had received Jennerian vaccination during pregnancy were refractive to vaccine virus during the first days of life, while Polano (7) demonstrated the transfer of tetanus antitoxin from mother to infant Bennholdt-Thomsen (8) showed that, in rabbits, immunization during pregnancy with *H pertussis* vaccine resulted in a passive transfer of complement-fixing antibodies to the offspring He concluded further that this transfer takes place particularly during the latter part of pregnancy, but not during nursing He was unable to immunize young rabbits actively until they reached the age of five weeks No reference has been noted of an attempt to apply this finding to human beings, but Weichsel and Douglas (9), using the complement-fixation test, and Bradford and Slavin (10) using the opsonocytopbagic test, have demonstrated a suggestive correlation between the level of *H pertussis* antibodies in the blood of mother and infant. Furthermore, Bradford (11) has reported a prophylactic effect from giving the blood of an individual convalescent from pertussis to a child recently exposed to the disease He regards this effect as being due to the establishment of a passive immunity analogous to that produced by the placental transfer of antibodies

[1] This investigation was aided in part, by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry American Medical Association.

[2] Working under a grant from the Fluid Research Fund of the University of Rochester

TABLE II

The opsono cytophagic reaction of the blood of mothers and newborns when the mother had a history of previous pertussis but no immunization during pregnancy

Family name	Mothers			Newborns		
	Distribution of cells according to number of organisms phagocytosed			Distribution of cells according to number of organisms phagocytosed		
	0 to 4 organisms	5 to 19 organisms	20+ organisms	0 to 4 organisms	5 to 19 organisms	20+ organisms
Do	0	6	19	0	12	13
Ma	0	5	20	1	1	23
Te	0	4	21	0	4	21
Le	0	14	11	0	15	10
Ni	0	3	22	0	3	22
Br	0	0	25	0	4	21
Ma	0	0	25	0	10	15
Co	0	0	25	1	9	15
Em	0	10	15	0	8	17
Fl	0	6	19	2	11	12
Mi	0	3	22	2	19	4
Average	0	4 6	20 4	0 5	8 7	15 7

phagic "index" in the present paper It should not be confused with the term as used by other authors A composite picture of the opsono-cytophagic "index" of the majority of blood samples tested is shown in Figure 1 Each column represents a mother and her infant whose "indices" have been superimposed For the sake of uniformity, the value for each immunized mother (Groups III and IV) represents the opsono-cytophagic reaction of her blood before she received vaccine In most instances it will be noted that the "index" for the mother exceeds that of the respective infant, but in several cases this value for the newborn is higher than its mother's The columns representing these exceptions are marked at the top by a plus sign They occur most frequently in Group IV where they comprise one-third of the columns The means of the "indices" for the mothers and the newborns of each group are shown by heavy horizontal bars, marked M A and N A, respectively The numerical values for these means are shown in Table V, which also contains the standard deviation and the probable error of each

Because the separation of the subjects into the four groups makes the number in each rather small, combinations of the mean "indices" of the groups were used This was done in an effort to increase the amount of data which could be used to demonstrate the effect of either the immunization (Group I plus Group II *vs* Group III plus Group IV) or previous pertussis in the mother (Group I plus Group III *vs* Group II plus Group IV) The average values for the mean "indices" in the combinations mentioned

TABLE III

The opsono-cytophagic reaction of the blood of mothers and newborns when the mothers had no history of pertussis but were immunized with a pertussis vaccine during pregnancy

Family name	Mothers Before Immunization			Total vaccine	Number of injections	Mothers After Immunization			Newborns		
	Distribution of cells according to number of organisms phagocytosed					Distribution of cells according to number of organisms phagocytosed			Distribution of cells according to number of organisms phagocytosed		
	0 to 4 organisms	5 to 19 organisms	20+ organisms			0 to 4 organisms	5 to 19 organisms	20+ organisms	0 to 4 organisms	5 to 19 organisms	20+ organisms
				cc							
Al	0	13	12	1 5	2	0	3	22	0	12	13
Be	0	3	22	1.5	2	0	3	22	0	7	18
Ho	0	16	9	1 5	3	0	15	10	0	6	19
Ug	0	6	19	2 0	3	0	8	17	0	13	12
Ho	0	17	8	1.5	2	0	9	16	7	18	0
Pi	0	5	20	1 0	2	0	5	20	14	9	2
La	0	4	21	3 5	4	0	9	16	0	9	16
Lo	0	3	22	1,0	2	0	8	17	4	6	15
Na	0	15	10	1.5	2	0	14	11	1	14	10
Fr	0	0	25	2.5	3	0	0	25	0	0	25
Ga	0	1	24	2 0	3	0	0	25	0	4	21
Average	0	7 6	17 4	1 8	2.5	0	6 7	18 3	2 4	8 9	13 7

TABLE IV

The opsono-cytophagic reaction of the blood of mothers and newborns when the mothers had a history of pertussis and also were immunized with a pertussis vaccine during pregnancy

Family name	Mothers Before Immunization			Total vaccine	Number of injections	Mothers After Immunization			Newborns		
	Distribution of cells according to number of organisms phagocytosed					Distribution of cells according to number of organisms phagocytosed			Distribution of cells according to number of organisms phagocytosed		
	0 to 4 organisms	5 to 19 organisms	20+ organisms			0 to 4 organisms	5 to 19 organisms	20+ organisms	0 to 4 organisms	5 to 19 organisms	20+ organisms
				cc.							
Cr	0	9	16	4.5	5	0	10	15	0	6	19
Al	0	1	24	2.0	3	0	1	24	0	0	25
De.	1	7	17	2.5	5	0	4	21	0	8	17
Me.	0	2	23	3.0	4	0	0	25	0	15	10
Li	0	8	17	1.5	3	0	4	21	0	7	18
Ru	0	24	1	2.5	3	0	21	4	1	10	14
Mu	0	15	10	1.0	2	0	15	10	1	17	7
Fr	0	15	10	1.0	2	1	12	12	0	10	15
Sr	0	17	8	2.5	3	0	1	24	0	11	14
Vo	0	8	17	1.5	3	0	1	24	1	7	17
Sm	0	5	20	1.0	2	0	0	25	0	0	25
De.	0	0	25	2.0	3	0	5	20	0	1	24
Pi	0	3	22	3.5	4	0	3	22	0	1	24
La	0	0	25	3.0	4	0	2	23	0	4	21
Hu	0	0	25	2 0	3	0	1	24	0	3	22
Av	0	1	24	2 0	3	0	2	23	0	14	11
Ca	0	3	22	2.5	3	0	0	25	1	4	20
Average	0	7	18	2.2	3	0	4.8	20.2	0 2	7	17.8

TABLE V

A summary of the mean opsono-cytophagic indices" (M) of both the mothers and the newborns in Groups I to IV, with the standard deviation (S D) and the probable error of the mean (P E M) of each

Group	Number of cases	Mothers						Newborns		
		Before immunization			After immunization			M.	S.D.	P.E.M.
		M.	S.D.	P.E.M.	M.	S.D.	P.E.M.			
I	11	18	7.89	1 689				9.0	4.71	1.005
II	11	20.4	4.13	0.696				13.7	6.61	0.945
III	11	17 4	6.46	1.38	18.3	4.82	1.025	13.7	7.29	1.55
IV	17	18	6.86	1.16	20.3	6.03	1.017	17.8	6.61	0.930

above are shown in Table VI A study of this table shows that the mother's previous pertussis and the artificial immunization each produce a statistically significant effect on the opsono-cytophagic "index" of the newborn s blood. It is obvious from Figure 1 that when these factors are combined (subjects of Group IV), a summation-effect is obtained which raises the mean "index" of the newborn to practically the same value as the mean 'index' of the mother

It is also apparent in Tables V and VI that neither of these two factors materially alters the opsono-cytophagic "index" of the mother In fact, the mean "index" of the 28 immunized mothers was changed so little by this procedure that the values before and after serve as a check on the reliability of the method for testing For this reason, in Figure 1 we feel justified in including only the "index" of blood samples taken from these mothers (Groups III and IV) before immunization was carried out

It is, of course, possible that the phagocytic power (as an expression of antibody titer) of each mother's blood exerts an influence on this capacity of her infant's blood, irrespective of whether she had previous pertussis or whether she was immunized In other words, one might expect to find a certain basic degree of correlation between mother and offspring The possible existence of this relationship was tested by means of a correlation graph, as shown in Figure 2 The data used in constructing this graph were the opsono-cytophagic "indices" of the 22 control (non immunized) mothers and newborns in our own group of subjects plus a group of 22

TABLE VI

'arious combinations of the data of Table V used to increase the number of cases influenced by either the history of previous pertussis for the mother or immunization of the mother during pregnancy

Groups	Number of cases	Qualification	Mothers		Newborns			Difference of means
			Before Immunization	After Immunization	M	S D	P E.M	
			M	M				
and II	22	Mother not immunized	19 2*		12 4	6 34	935	3 8 ± 1 23
II and IV	28	Mother immunized	17 7*	19 4*	16 2	6 50	845	significant
and III	22	No pertussis for mother	17 7*		11 4	6 48	915	5 6 ± 1 21
I and IV	28	Mother had pertussis	18 9*		17	5 64	583	significant

* Means are so close that calculation of S D and P E M is not worth while

omparable pairs of mother and offspring taken rom the previously published results of Brad- ord and Slavin (10) Since this latter group ras tested in the same manner by the same person vho performed our tests, it seemed proper to include them Of the 44 mothers used for this purpose, 25 had had pertussis and 19 had not Although it does not appear striking to the eye, a statistical analysis of the data comprising this graph shows a significant correlation to be present

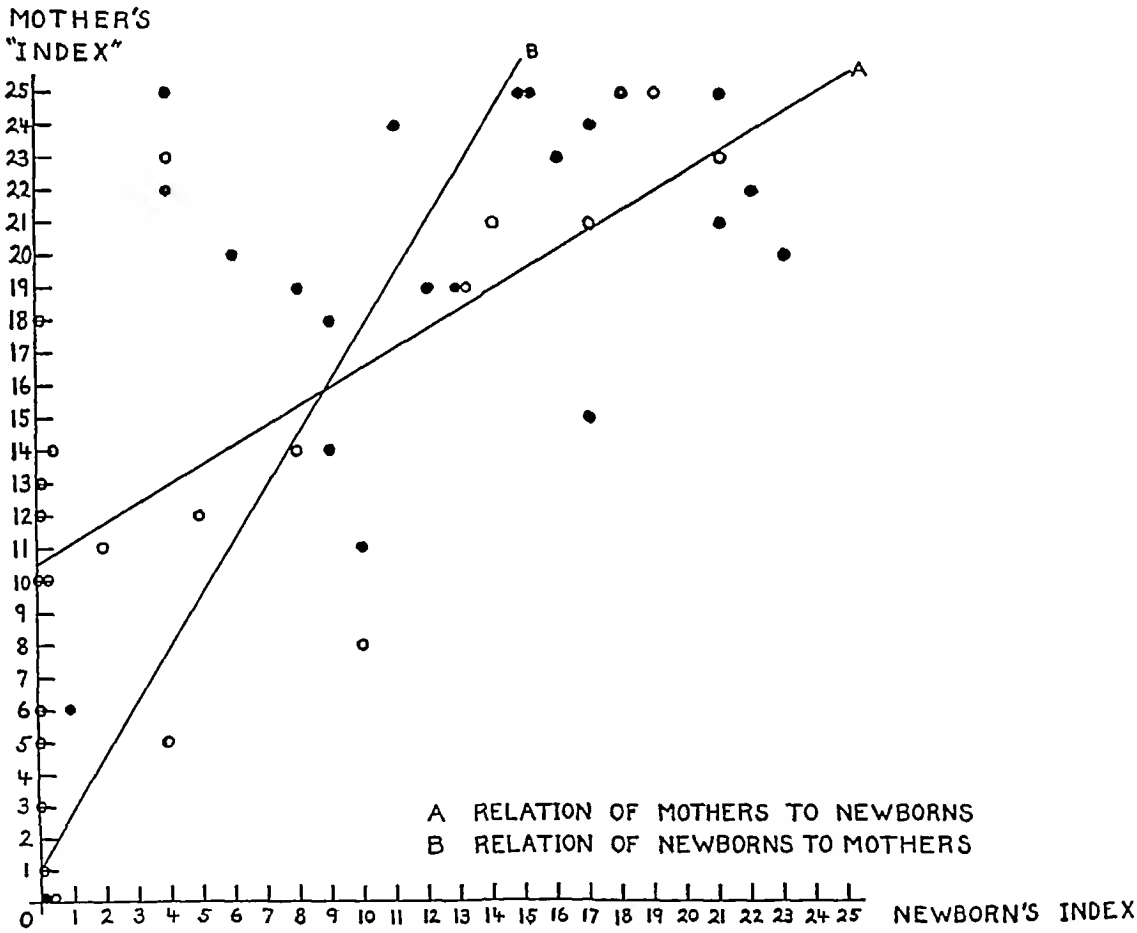


FIG 2 CORRELATION GRAPH FOR THE OPSONO-CYTOPHAGIC "INDEX" OF MOTHER AND NEWBORN

● Series I (22 cases)
○ Series II (22 cases)

between the "indices" of the mother and of the newborn

In Table VII we have arranged the results of the opsono-cytophagic reaction of the bloods of 5 newborns tested before the first period of nursing and again at the end of the first week of life It is clear that there has been practically no change in this reaction, certainly no increase in phagocytic capacity, after receiving colostrum

TABLE VII

The effect of colostrum on the opsono-cytophagic reaction of the newborn's blood

Family name	Newborn before nursing			Newborn after nursing one week		
	Distribution of cells according to number of organisms phagocytosed			Distribution of cells according to number of organisms phagocytosed		
	0 to 4 organisms	5 to 19 organisms	20+ organisms	0 to 4 organisms	5 to 19 organisms	20+ organisms
Li	0	7	18	0	7	18
Be.	0	7	18	0	8	17
Ho	0	6	19	0	5	20
Ru	1	10	14	2	20	3
Hod.	7	18	0	16	9	0

DISCUSSION

Bradford and Slavin (10) concluded from their studies of the opsono-cytophagic reaction of the blood of mothers and newborns that (*1*) there was a certain degree of correlation between their respective titers, and (*2*) that the titer of both mother and newborn was higher when the former had had pertussis Our results are in accord with the first of these conclusions and with a part of the second, namely, that the mother's previous pertussis exerts a definite influence on the opsono-cytophagic reaction of the newborn's blood. We are not able to show that it affects the titer of the mother's blood to any significant degree. We are unable to explain this discrepancy

Our results correspond with what one would expect on the basis of the studies of Bennholdt-Thomsen (8), demonstrating the trans placental passage of complement-fixing antibodies from rabbits immunized with a pertussis vaccine to their offspring They are not in accord with the conclusion of Kendrick, Gibbs and Sprick (16) that the blood of newborn infants shows a negative or very weak opsono-cytophagic reaction regardless of the reaction of the mother

Our results suggest that at least three factors may exert an appreciable influence on the phagocytic capacity of the blood leukocytes of the newborn infant for *H pertussis* (*1*) The phagocytic power of the mother's blood, (*2*) previous pertussis in the mother, and (*3*) artificial immunization of the mother with pertussis vaccine during the latter part of pregnancy There may be many additional factors By a satisfactory grouping of our subjects we can study the effect of the second and third factors independently of each other, but the effect of the first factor obviously cannot be eliminated by this means Therefore, it seems best to consider the phagocytic power of the newborn's blood in terms of the same capacity of the mother's blood If we apply this line of reasoning to Figure 1, the mean "index" of the newborns in Group I is 50 per cent of that of the respective mothers while in each of Groups II and III it is approximately 75 per cent and in Group IV it has risen to 100 per cent (*i e*, equal to that of the respective group of mothers) One might be justified in concluding that either previous pertussis in the mother or active immunization during pregnancy increases this phagocytic capacity of the newborn to a similar degree, and both factors exert a summation-effect which puts the newborn at the same level as the mother

Three important questions arise (*1*) Is the phagocytic reaction a true measure of the specific opsonizing antibodies for *H pertussis* in the newborns studied? (2) How long during early infancy will it remain unchanged? (*3*) Is there any correlation between this capacity and resistance to pertussis? In answer to the first question Bradford and Slavin (10), Kendrick *et al* (16) and Singer-Brooks and Miller (15) have shown that the phagocytic capacity increases in the blood of patients during the latter part of pertussis and during convalescence Furthermore, Bradford *et al* (17) have shown that it increases in infants and children following the injection of anti pertussis serum (human or rabbit), and Kendrick *et al* (16) have found that it is increased in human beings by active immunization with a pertussis vaccine. However, Singer-Brooks and Miller (15) have shown it ir

creased in subjects after immunization with a nonspecific, "mixed respiratory" vaccine, and they present a detailed discussion of some of the nonspecific factors which may influence phagocytosis of organisms by blood leukocytes

We are unable to give a definite answer to either the second or the third questions because of the small number of patients in our study, and because we were unable to get a sufficient number of subsequent opsono-cytophagic tests on our infants during their first year of life to justify any conclusions However, it occurred to us that some clinical evidence could be obtained from the hospital records which might furnish a partial answer to both these questions Thus, if this laboratory test be a measure of resistance to pertussis, during the first half-year of life infants whose mothers had had pertussis might be expected to have milder cases of the disease than infants whose mothers had not had this infection After the age of 6 months this effect might be expected to disappear as the titer of "inherited" antibodies decreased in the blood of the child, provided one can reason by analogy from other infectious diseases, such as diphtheria One simple way to test this theory is to note the effect of the mother's previous pertussis on the infant mortality rate from this disease Accordingly, the family histories of 45 infants known to have died from pertussis in this hospital were studied Definite information could be obtained in only 31 of these, but in the cases of 18 infants who died before the age of 6 months, the mothers of only 5 had had previous pertussis, while in the group of 13 infants who died after the age of 6 months, 7 of the mothers had had pertussis and 6 had no knowledge of it In other words, the findings seemed to bear out the argument advanced above The results are strengthened by the fact that the observations were made on people of the same geographical and economic position as that group of mothers who were vaccinated in our own studies, and in this latter group (selected at random) more than half of the mothers had had pertussis Only in that group of mothers whose infants died from pertussis under the age of 6 months do we find any appreciable decrease in the incidence of this disease The series of cases is too small to permit definite conclusions, but the evidence is in agreement with the contention that resistance to pertussis in infancy may be associated with the phagocytic reaction of the blood for *H pertussis*

It seemed surprising that the mean "index" of the immunized mothers did not increase as a result of this procedure We have no adequate explanation for this finding, except that this value was in general quite high before immunization was started

In the five infants tested before and after nursing, the results suggest that the colostrum has no effect upon the phagocytic reaction of the newborn However, we do not feel justified in drawing conclusions from such a limited experience Possibly the interval between the two tests should be lengthened

SUMMARY

1 The opsono-cytophagic reaction of the blood of the newborn infant for *H pertussis* is significantly influenced by at least three factors, (*a*) the phagocytic capacity of the mother's blood, (*b*) previous pertussis in the mother, and (*c*) artificial immunization of the mother with pertussis vaccine during the latter part of pregnancy

2 Certain evidence from hospital case records suggests that this phagocytic capacity may be regarded as a measure of resistance to pertussis

3 Neither the previous disease nor artificial immunization with a pertussis vaccine during pregnancy exerted any significant influence on the opsono-cytophagic reaction of the mother's blood for *H pertussis*

4 In a few infants tested before and after nursing, no apparent increase in the phagocytic capacity of blood for *H pertussis* was produced by the colostrum obtained during the first week of life

We are greatly indebted to Dr S W Clausen for his statistical analysis of the data, and to Dr Karl Wilson for permission to use the patients on the Obstetrical Service of the Strong Memorial and Rochester Municipal Hospitals Dr G P Berry assisted in the preparation of this report with many valuable suggestions

BIBLIOGRAPHY

1 McKhann, C. F, and Chu, F T, Antibodies in placental extracts J Infect. Dis, 1933, 52, 268

2 Ratner, B, Jackson, H C., and Gruehl, H L., Transmission of protein hypersensitiveness from mother

to offspring I. Critique of placental permeability J Immunol., 1927 14, 249
3 Aycock, W L. and Kramer, S D., Immunity to poliomyelitis in mothers and the newborn as shown by the neutralization test. J Exper Med., 1930, 52, 457
4 Pfaundler, M., and Schlossmann, A The Diseases of Infants and Children. Vol. III (Translated by M G Peterman.) J B Lippincott Co., Philadelphia, 1935 4th ed.
5 Griffith, J P C. and Mitchell, A. G, The Diseases of Infants and Children. W B Saunders Co., Philadelphia, 1937, new 2d ed.
6 Burckhardt, A. E. Zur intrauterinen Vaccination. Deutsches Archiv f klin. Med., 1879, 24, 506
7 Polano, O., Der Antitoxinubergang von der Mutter auf das Kind. Ein Beitrag zur Physiologie der Placenta Ztschr f Geburtsh. u Gynäk., 1904, 53, 456
8 Bennholdt Thomsen, C., Das Verhalten eines gegen den Bordet Gengou Bacillus spezifischen Ambo ceptors bei Mutter und Kind Ztschr f Kinderh., 1934–35, 57, 532.
9 Weichsel, M., and Douglas, H. S., Complement fixation tests in pertussis. J Clin. Invest. 1937 16, 15
10 Bradford W L. and Slavin, B., The opsono-cytophagic reaction of the blood in pertussis J Clin. Invest. 1937, 16, 825
11 Bradford, W L., Use of convalescent blood in whooping cough, with a review of the literature. Am. J Dis Child., 1935 50, 918.
12. Rodolfo, A., A study of the permeability of the placenta of the rabbit to antibodies. J Exper Zool., 1934 68, 215.
13 Sauer L. W., Immunization with bacillus pertussis vaccine. J A. M. A., 1933 101, 1449
14 Huddleson, I F., Johnson, H W., and Hamann, E. E., A study of the opsono-cytophagic power of the blood and allergic skin reaction in Brucella infection and immunity in man. Am. J Pub Health, 1933 23 917
15 Singer Brooks C., and Miller J J., Jr., The opsono-cytophagic test in children with pertussis and in children vaccinated with *H pertussis* antigens J Clin. Invest., 1937, 16, 749
16. Kendrick, P., Gibbs J and Sprick, M., The opsono-cytophagic test in the study of pertussis J Infect. Dis., 1937, 60, 302.
17 Bradford, W L., Mikell, R., and Slavin, B., The effect of immune blood upon the opsono-cytophagic power of the blood in pertussis. J Clin Invest. 1937, 16, 829

TABLE I

Group I

ıtion ıding ıritis	Onset of nephritis	Urinary findings	Hemolytic streptococcus recovered	Maximum antistreptolysin titer	Date of healing	Subsequent base of antistreptolysin titer	Duration of nephritis	Interval between healing and next hemolytic streptococcal infection	Subsequent hemolytic streptococcal invasion	Hemolytic streptococcus recovered	Maximum antistreptolysin titer	Urinary findings associated with subsequent hemolytic streptococcal invasion
media, ditis, ricular s	Apr 30, 1934	Alb. ++++ Many RBC and casts	From postauricular abscess May 1 1934	333 May 3, 1934	July 11, 1934	71 Dec. 5, 1934	2½ mos.	9 mos., with 5 neg. urine exams.	Apr 4, 1935, bilateral otitis media, pharyngitis, postauricular abscess, erysipelas	From postauricular abscess Apr 10, 1935	1250 Apr 5, 1935	Alb. +, sediment neg throughout 6 wks. 8 neg. urine exams. over next 5 mos.
ral media, al is	Feb 10, 1934	Alb. ++++ Gross hematuria, many casts	From throat Mar 8, 1934	333 Mar 14, 1934	May 23, 1934	11 June 13, 1934	3 mos.	17 mos., with 17 neg. urine exams.	Oct. 30, 1935 common cold, pharyngitis	From throat Nov 1, 1935	500 Nov 13, 1935	Alb +, sediment neg. for 2 days, 6 neg. urine exams. over next 6 mos.
ngitis, media	Jan. 1, 1934	Alb. ++++ Many RBC and casts	From mastoid Jan. 6, 1934	333 Jan. 10, 1934	Aug. 15, 1934	16 Sept. 26, 1934	7 mos.	9 mos., with 5 neg. urine exams.	May 20, 1935, cervical adenitis	From throat June 8, 1935 none June 11, 1935 none June 16, 1935 none	500 June 11, 1935	Alb. + with sediment neg. for 12 days, 5 neg. urine exams. in next 2 mos.
cold ever	June 19, 1933	Alb ++++ Gross hematuria, many casts	From throat June 22, 1933	500 July 3, 1933	Mar 7, 1934	83 June 13, 1934	9 mos.	9 mos., with 2 neg. urine exams.	Dec. 28, 1934, "common cold"	From throat Jan. 3, 1935	830 Jan. 23, 1935	Alb. + in 1 spec sediment neg 1 wk. later and over next 3 mos. 3 neg. urine exams.
ngitis, media, ditis	Apr 1, 1934	Alb. ++ Many RBC and casts	From ear Mar 21, 1934, from mastoid Apr 2, 1934	1000 Apr 16, 1934	Apr 22, 1934	166 Jan. 3, 1935	3 wks.	3 yrs., with 10 neg urine exams. and 1 showing orthostatic albuminuria (see protocol)	Mar 21, 1937 head cold, cough, T 101°	From throat Apr 7 1937 Apr 14, 1937	333 Apr 7, 1937	Urine neg except 1 occasion of probable orthostatic albuminuria (see protocol) 5 urine exams over next mo revealed only orthostatic albuminuria
ngitis, media	Mar 24, 1934	Alb ++++ Gross hematuria, many casts	From throat and draining mastoid Mar 26, 1934	1000 Apr 12, 1934	June 6, 1934	71 Feb 27, 1935	2½ mos.	10 mos., with 6 neg urine exams.	Apr 17 1935, acute mastoiditis	From throat, draining ear, and postauricular abscess Apr 17, 1935	333 May 16, 1935	Alb + for 4 days in hospital, 1 mo later urine neg. (pt. lost to follow up)
ngitis, al is, nasillar s	June 13, 1933	Alb ++++ Gross hematuria, many casts	From peritonsillar abscess June 15, 1933	250 July 20, 1933	Nov 22, 1933	83 Nov 22, 1933	5 mos.	18 mos., with 2 neg. urine exams. (1st and 18th mo.)	Cervical adenitis "all winter" May 15, 1935, acute cervical adenitis	No culture made	833 May 22, 1935	1 urine exam. 1 wk. after acute infection neg. (pt. lost to follow-up)
ngitis, al is	June 15, 1934	Alb. ++++ Few RBC and casts	From throat May 27, 1934 from cervical abscess June 18, 1934	555 June 25 1934	Aug. 1, 1934	50 Apr 27, 1935	1½ mos.	8 mos., with 15 neg. urine exams.	Apr 25, 1935 pharyngitis, cervical adenitis May 1, 1935 erythema nodosum May 3, 1935, bronchopneumonia	From throat Apr 25 1935, from sputum May 3, 1935	333 May 14, 1935	Neg on 15 occasions over 1 mo

TABLE II
Group II

Case, hospital number, sex, and age	Infection preceding nephritis	Onset of nephritis	Urinary findings	Hemolytic streptococcus recovered	Maximum antistreptolysin titer	Date of healing	Subsequent base of antistreptolysin titer	Duration of nephritis	Interval between healing and next hemolytic streptococcal infection	Subsequent hemolytic streptococcal invasion	Hemolytic streptococcus recovered	Maximum antistreptolysin titer	Urinary findings associated with subsequent hemolytic streptococcal invasion
P. F. K. 335068 ♂ Age 14	Pharyngitis, peritonsillar abscess, T. 104.6°	Apr. 18, 1922	Alb. ++++ Gross hematuria, many casts	From pus of peritonsillar abscess	625 Apr. 25, 1922	Mar 14, 1923	33 Jan. 10, 1923	1 mo.	10 mos. 24 urinary tests, 12 in 1st 6 wks., 3 on subsequent examinations	Cold with cervical adenitis for 1 wk. Mar 27 1923	None recovered from throat 2 wks. after onset of infection	333 May 16, 1923	Gross hematuria noted by pt. 1 wk. before visiting clinic and progressively clearing over the following month. Subsequently over 3 yr. period 11 urine specimens showed no abnormalities
10. J. L. 242223 ♂ Age 18	Lobar pneumonia Type I	Aug. 2, 1924	Alb. ++++ Smoky urine, many casts	None	Not done	Nov 18, 1925	Not done	15 mos.	10 yrs. with 8 negative urines	Pharyngitis, cervical adenitis, T. 101 May 6, 1936	None recovered on throat culture	Not determined	Alb. ± gross hematuria. 1 wk. later no hematuria. 1 yr later alb. ± sediment neg.

which time urine specimens revealing normal findings were observed on 17 occasions The antistreptolysin titer had declined to 50 at the time of examination of the first normal urine. Seventeen months after the healing of his nephritis, the boy was readmitted with a head cold and pharyngitis. Hemolytic streptococci were cultured from the throat, and the antistreptolysin titer reached 500 units. The urine showed minimal amounts of albumin and a normal sediment over the 2 days during which fever was present. There were no clinical signs of nephritis. The urine then became negative and remained so over a 6-month period of observation in the clinic, during which time there were monthly urine examinations.

Case 3 T B (Hospital Number 403728)

A boy of 6 with a month s preceding history of pharyngitis and otitis media, was admitted with acute nephritis. There was no edema or hypertension. Blood nonprotein nitrogen was 39 mgm. per 100 cc. The urine showed albumin ++++ many red blood cells and casts Hemolytic streptococci were cultured from the mastoid at operation, and the antistreptolysin titer reached 333 units

Course The urine cleared progressively, showing only albumin ++ and 1 to 2 red blood cells at the end of 2 weeks, which minor findings persisted until they completely disappeared at the end of 7 months. At this time, the antistreptolysin titer had declined to 16 units. During the next 9 months there were 5 urine examinations all revealing normal findings. At the end of this time, the patient was readmitted with a history of cervical adenitis for 9 days, fever having reached 104 F Throat cultures on 3 occasions failed to show hemolytic streptococci but the antistreptolysin titer was 500 units. The urine showed minimal amounts of albumin over a 12-day febrile period There were no clinical features of nephritis. Five urine examinations over the next 2 months were normal.

Case 4 B G (Hospital Number 379237)

A boy of 6 with a history of head cold and fever for 2 weeks was admitted with acute nephritis, gross hematuria having been noted 3 days before admission. There was no edema, blood pressure was 104/60 and blood nonprotein nitrogen was 59 mgm. per 100 cc. The urine showed albumin ++++ and casts in addition to the gross hematuria. Hemolytic streptococci were cultured from the throat. The antistreptolysin titer reached 500 units.

Course The urine improved progressively showing albumin ++ and 3 to 4 red blood cells per high power field at the end of 6 weeks when he was discharged. After 2 months, he was readmitted with an otitis media, his urine still showing albumin + and 1 to 3 red blood cells per high power field. The otitis media subsided in a week and the urine cleared progressively becoming normal 9 months from his first admission. During the following 9 months, a urine examination upon 2 occa

sions revealed normal findings and the antistreptolysin titer was 111 units At this time, the boy was seen in the clinic with a cold Temperature was 103° F Hemolytic streptococci were cultured from the throat and the antistreptolysin titer reached 830 units There was no clinical evidence of nephritis The urine showed minimal amounts of albumin and a negative sediment on admission One week later, and on 3 occasions over the next 3 months, the urine was negative.

Case 5 N S (Hospital Number 410777)

A boy of 5 with a 2 weeks' preceding history of pharyngitis and otitis media was admitted with acute mastoiditis and acute nephritis There was no edema, blood nonprotein nitrogen was 31 mgm. per 100 cc., and blood pressure was 118/65 The urine showed albumin ++, many red blood cells, and many casts Hemolytic streptococci were cultured from the mastoid at operation and had been cultured from a draining ear 10 days before, during a clinic visit. The antistreptolysin titer reached 1000 units

Course The urinary findings cleared progressively over the next 3 weeks and were normal at the end of this time. Subsequently, in the clinic, over a period of 12 months, 8 urine examinations revealed normal findings, and the antistreptolysin titer had declined to 144 units At the next visit, 3 months later, the boy reported fever for 2 days following swimming He complained of general muscle stiffness There had been some dysuria but no frequency or hematuria Physical examination revealed no signs of nephritis No throat culture was taken The urine showed albumin ++++ and a normal sediment The antistreptolysin titer was 200 Ten days later, a urine specimen was normal The boy was next seen in the clinic approximately 1½ years later, reporting a severe head cold and cough, fever having reached 101° F over the preceding week. Hemolytic streptococci were cultured from the throat on two occasions and the antistreptolysin titer was 333 units The urine showed only heavy albuminuria proven to be an orthostatic albuminuria on 5 examinations during the subsequent month There were no clinical signs of nephritis

Case 6 J L (Hospital Number 410752)

A boy of 4 with a 2½ weeks' history of pharyngitis, otitis media, and fever 102° F, was admitted with acute nephritis There was no edema, blood nonprotein nitrogen was 36 mgm. per 100 cc., and blood pressure was 95/55 The urine showed gross hematuria, albumin ++++, and many casts Hemolytic streptococci were cultured from the throat, draining ears, and from the mastoid area at operation. The antistreptolysin titer rose to 1000 units

Course The urine cleared progressively, becoming completely normal in 2½ months Ten months later, during which period urine examinations upon 6 occasions revealed no abnormality, the patient was readmitted with acute mastoiditis Temperature was 101° F There was no clinical evidence of nephritis Hemolytic streptococci were cultured from the throat, ear, and from a postauricular abscess The antistreptolysin titer reached 333 units The urine showed a minimal albuminuria and a normal sediment daily during the 4 days that the patient was hospitalized. The urine 1 month later was normal The patient was then lost to follow-up

Case 7 A V (Hospital Number 379219)

A boy of 7 with a history of pharyngitis and cervical adenitis for 2 weeks was admitted with a peritonsillar abscess and acute nephritis with gross hematuria There was slight edema of the extremities, blood pressure was 116/68, blood nonprotein nitrogen 31 mgm per 100 cc, serum albumin 3 9 per cent, and serum globulin 3 4 per cent. In addition to gross hematuria, the urine showed albumin ++++, and casts Hemolytic streptococci were cultured from the peritonsillar abscess, and the antistreptolysin titer reached 250 units

Course The urinary abnormalities subsided gradually, showing albumin + and occasional red blood cells at the end of 6 weeks At the end of 5 months the urine was completely normal and the antistreptolysin titer revealed 83 units Eighteen months after the date of healing, during which time urine examinations in the 1st and 18th months revealed normal findings, the child was seen in the clinic with the complaint of cervical adenitis "off and on all winter," a recent exacerbation with fever having occurred one week previously No clinical signs of nephritis were present No throat culture was made, but the antistreptolysin titer was found to be 833 units The urine at this time, one week after exacerbation of an infection with fever, revealed no changes Unfortunately, the patient could not be followed.

Case 8 R M (History Number 419533)

A girl of 21 was admitted with pharyngitis and cervical adenitis The urine was normal on admission and remained so during a period of 10 days of exacerbation and remission of the adenitis Throat cultures revealed hemolytic streptococci and the antistreptolysin titer reached 555 units Ten days later, the urine began to show ++++ albumin, a few casts, and red blood cells There was no edema and no hypertension, but the blood urea rose to 0 79 gram per liter The adenitis persisted and surgical drainage of a cervical abscess revealed, on culture, the presence of hemolytic streptococci Blood cultures showed no growth. The urinary abnormalities gradually diminished and at the end of 6 weeks the urine was normal Eight months after healing had occurred, during which period there had been 15 urine examinations, all normal, and the antistreptolysin titer had declined to 50 units, the patient was readmitted with pharyngitis and cervical adenitis of 12 hours' duration Temperature was 101° F Throat culture showed a pure growth of hemolytic streptococci The antistreptolysin

titer rose to 333 units Six days after admission, the patient developed mild erythema nodosum of the left foot and leg and right forearm Eight days after admission, she developed a bronchopneumonia with sputum showing hemolytic streptococci and no pneumococci. Throughout this second admission, over a period of 1 month there were 15 urine examinations all of which were normal. Fever was high, 101° to 104° F over the first week of illness. There were no clinical signs of nephritis

GROUP II

Case 9 F K (Hospital Number 336058)

A boy of 14 with a 2 weeks' history of pharyngitis was admitted with a peritonsillar abscess and acute nephritis with gross hematuria. Temperature was 104.6 F., and blood pressure 134/64 There was no edema. The urine, in addition to gross hematuria, showed albumin ++++ and many casts. Hemolytic streptococci were cultured from the incised peritonsillar abscess and the antistreptolysin titer reached 555 units. The patient left against advice after 3 weeks in the hospital, at which time the urine showed albumin ++ and rare red blood cells Five days later, when seen in the clinic, the urine showed albumin ++ and 10 red blood cells per high power field. There was no history of intercurrent infection. When readmitted 1 week later, the urine was normal and remained so throughout his stay of 2 months During this time, the antistreptolysin titer had declined to 200 Throughout the next 6 months, 3 more urine examinations revealed normal findings Ten months from the date of healing the patient returned to the clinic, reporting a cold and cervical adenitis for 1 week, gross hematuria having appeared on the 2d day A urine examination in the clinic revealed albumin +, many red blood cells and no casts. There was no edema, and blood pressure was 130/95 A throat culture at this time failed to show hemolytic streptococci No determination of the antistreptolysin titer was made. Over the next 4 weeks, numerous red blood cells with albumin + and without casts were found in his urine on 3 examinations One month later an examination revealed normal urine. During the next 3 years there were 15 urine examinations revealing normal findings.

Case 10 J L. (Hospital Number 242232)

A boy of 16 was admitted with Pneumococcus Type I lobar pneumonia of 3 days' duration. Urine on admission showed albumin +, occasional casts, and rare red blood cells. Blood pressure was 100/65 Three days later, his eyes became puffy blood pressure 130/65 and the urine became smoky Albumin ++++ was present. Blood urea rose to 1.6 grams per liter and the phthalein excretion fell to 25 per cent He was discharged 3 months later with urine still showing albumin ++ and many red blood cells. Subsequent follow up in the clinic 1 month later showed albumin + and a few red blood cells per high power field and 1 year later albumin ± with a normal sediment. During the next 10 years, he was followed in the clinic, the urine being negative on 8 occasions. At the end of this 10 year period the patient returned to the clinic with sore throat, dysphagia, cervical adenitis, and temperature 101° F, blood pressure was 115/80 and there was no edema. Urine showed albumin ± and 35 red blood cells per high power field. A throat culture revealed no hemolytic streptococci but the antistreptolysin titer was found to be 333 units. During the next week, the patient's symptoms disappeared. The urine 1 year later, showed albumin ±, sediment negative. The antistreptolysin titer was 333 units

SUMMARY

The case histories of two groups of patients with healed acute nephritis are presented The first group, offered in Table I, consists of 7 children and 1 adult. All of them had typical acute glomerulonephritis, associated at onset with an infection, bacteriologically and immunologically proved to be due to the hemolytic streptococcus The duration of the nephritis in these patients varied from 3 to 9 months There was a period of observation during the healed state ranging from 9 months to 3 years All of the patients suffered subsequent infection, again bacteriologically or immunologically proved to be due to the hemolytic streptococcus The antistreptolysin titers ranged from 333 to 1250 units There were no clinical symptoms of nephritis associated with the second hemolytic streptococcal invasions in this group of patients The urine showed no red blood cells and only an initial minimal and transient albuminuria associated with the febrile episodes Thereafter, throughout periods of observation varying from 1 to 6 months in 7 of these patients, the urine showed no changes distinguishable from the normal[1] Unfortunately, in the remaining patient (Case 7, A. V), only 1 urine was available for examination, 1 week after an attack of acute cervical adenitis The findings at this time, however, were entirely normal

One of the patients in this group (Case 5 N S) was of particular interest. During his healed period, he developed fever with mild dysuria, without hematuria or frequency A urine speci-

[1] It should be said that one of us J D L., has been able to find from time to time, with special technique, minimal amounts of albumin, occasional casts and red blood cells in the urine of these patients, as he does with the same technique in the urine of normal children.

men revealed albumin ++++ and occasional red blood cells Ten days later, the urine was normal One and a half years later, an examination, following a hemolytic streptococcal infection, showed that the boy had developed orthostatic albuminuria and was without evidence of nephritis In the light of this, one is unable to state whether the single specimen showing heavy albuminuria 1½ years previously, associated with fever and dysuria, and followed later by one urine revealing normal findings, was of the orthostatic type or whether it represented nephritis However, the second proved hemolytic streptococcal infection did not induce recurrence of the nephritis and 5 urine specimens observed over the next month continued to show only albuminuria of the orthostatic type.

The second group of patients consists of 2 adolescents presented in Table II This group differs from Group I inasmuch as it consists of individuals healed of acute glomerulonephritis, who, on being subjected to subsequent infection, developed marked hematuria In Case 9, F K, aged 14, the original bout of nephritis followed an infection proved to be due to the hemolytic streptococcus, in the other, Case 10, J L, aged 16, the nephritis followed Type I lobar pneumonia, and a concomitant infection with the hemolytic streptococcus was not known to be present The nephritis lasted only 1 month in Case 9 but abnormal urinary findings persisted 15 months in Case 10 Each suffered subsequent infection This was proved to be due to the hemolytic streptococcus in Case 10 and occurred 10 years after the healing of his nephritis The ensuing infection in the other patient, Case 9, F K, occurred 10 months after the healing of his nephritis and was presumably due to the hemolytic streptococcus but was not proved so As has been stated above, the feature distinguishing these two patients from the individuals in Group I was the development of marked hematuria concomitant with subsequent hemolytic streptococcal infection and following a period in which they had been amply observed, as will be seen by the tables, to have been healed of their nephritis The hematuria, although marked, was unaccompanied by significant albuminuria, it lasted 4 weeks in Case 9 and 1 week in Case 10 Subsequently, in Case 9, 15 urine examinations over the next 3 years revealed no abnormalities Only 1 examination has been obtained in Case 10 and that 1 year after the disappearance of his hematuria At this time, no urinary abnormalities were present

In connection with these 2 patients exhibiting hematuria without significant albuminuria, it is of interest to point out that about 15 per cent of the patients with rheumatic fever at the Presbyterian Hospital show varying degrees of hematuria and slight albuminuria during their acute episodes whereas, at autopsy, only 3 of about 100 patients in Coburn's series (5) dying of active rheumatism showed any evidence of glomerulonephritis It seems possible that the hematuria associated with the subsequent hemolytic streptococcal infection occurring in the two individuals in the second group of patients and unassociated with other signs, symptoms, or laboratory evidence of nephritis may be analogous to the renal manifestations occurring in many cases of rheumatic fever However, it must be stated that the mechanism of the hematuria is unknown

It is of interest immunologically that in acute nephritis, a disease the typical onset of which follows an infection with the hemolytic streptococcus, a second similar infection in these patients has not led to the chronic form of the disease. In the rheumatic state, however, a disease of proved association with the hemolytic streptococcus, recurrence of the disease, with or without apparent progressive damage, is common

If the 10 cases presented may be assumed to be representative of the disease in general and if acute glomerulonephritis be a disease initiated by the hemolytic streptococcus, the permanence of recovery maintained in the face of subsequent hemolytic streptococcal invasion, as evidenced by the 10 patients under discussion, presents one aspect of immunity in hemolytic streptococcal disease

The nature of this immunity is not known Whether the defense mechanism results from the actual presence of immune bodies or whether it may result from histological and physiological changes within the kidney such as MacNider (3, 4) described occurring in his dogs with uranium nitrate nephritis, who were resistant to further damage by uranium nitrate, can only be a matter for conjecture at present. The important fact to be emphasized again in connection with this series

of patients with healed acute nephritis is that, in spite of recurrent hemolytic streptococcal infection, and, in two instances, despite an accompanying recurrent hematuria, no one of these individuals has gone on to develop chronic progressive kidney disease.

The observations reported in this study receive amplification and support in the experience of Atchley and Loeb in the nephritic clinic at the Presbyterian Hospital over a period of 14 years and in the experience of one of us (J D L) at the Babies Hospital over a 10-year period No case of healed acute glomerulonephritis has subsequently been observed to develop the chronic form of the disease. These cases, for the most part, were not studied from the bacteriological and immunological standpoint, but were presumably, in the majority of instances, secondary to infection with the hemolytic streptococcus

CONCLUSIONS

1 A clinical, bacteriological and immunological study with reference to hemolytic streptococcal infection has been made in 10 patients These individuals were observed (1) during their acute glomerulonephritis, (2) throughout a subsequent healed period, and (3) during and following a subsequent infection with the hemolytic streptococcus

2 Eight of the patients whose nephritis at onset was preceded by hemolytic streptococcal infection were observed through healed periods varying from 8 months to 3 years Thereafter, in each instance, an intercurrent hemolytic streptococcal infection produced no recurrence of their nephritis

3 Two of the patients with acute glomerulonephritis, preceded by hemolytic streptococcal infection in one and by Type I lobar pneumonia in the other, were observed through subsequent healed periods of 10 months and 10 years respectively Each then underwent an infection proved to be caused by the hemolytic streptococcus in one instance and presumably caused by that organism in the other Both developed transient gross hematuria without significant albuminuria.

4 No one of the 10 patients studied has developed the chronic form of glomerulonephritis

BIBLIOGRAPHY

1 Berglund, H., Medes G., Huber, G C., Longcope, W T., and Richards, A. N , The Kidney in Health and Disease. Lea and Febiger Philadelphia, 1935, p. 346.

2. Boyle, H H., Aldrich C. A Frank, A., and Borowsky S , The Addis count in children following clinical recovery from postinfectious nephritis. J A. M. A., 1937 108, 1496.

3 MacNider W deB., The development of the chronic nephritis induced in the dog by uranium nitrate. A functional and pathological study with observations on the formation of urine by the altered kidneys J Exper Med. 1929, 49 387

4 MacNider, W deB., The functional and pathological response of the kidney in dogs subjected to a second subcutaneous injection of uranium nitrate. J Exper Med., 1929, 49 411

5 Personal communication.

COMMENT

Longcope (9) studied 36 adult patients during the acute stage of hemorrhagic nephritis Twenty-three patients or 64 per cent showed antistreptolysin titers above 125 units The highest titer observed was 500 units, found in 2 patients The type of infection accompanying the nephritis was quite different in Longcope's series from ours The deep infections such as mastoiditis and cervical lymphadenitis which occurred so frequently in our series where children predominate are not found in the group observed in Baltimore This may account for the smaller percentage of cases showing antistreptolysin elevation and the lack of very high antistreptolysin titers

Careful study of our clinical and immunological data indicate that in the acute stages of glomerulonephritis the maximum antistreptolysin titer is (*1*) not related to age or sex, (*2*) has no relation to the severity or duration of the nephritis, but (*3*) is definitely related to the type and severity of the acute infection which precedes or accompanies the acute nephritis

The data presented here establish the fact that in spite of the heterogeneous group of prodromal infections observed or the unsatisfactory history and clinical evidence of the preceding infection in many cases, 94 per cent of 116 consecutive cases of acute glomerulonephritis show specific immunological evidence of having had a recent hemolytic streptococcal infection

The titer of antistreptolysin following acute glomerulonephritis

The diagram in Figure 2 shows the variations in immune response during the two years following the onset of acute nephritis This diagram was made by using only the highest antistreptolysin titer found for each patient in each of the first twelve months after onset For the second year after the onset, all the antistreptolysin determinations on each patient studied are included The majority of the 63 patients in this group had from 4 to 8 antistreptolysin determinations during this 2-year period

The diagram shows that in the second month the number of normal titers begins to increase and the number of high titers decreases In the second year after onset the titers in 75 per cent

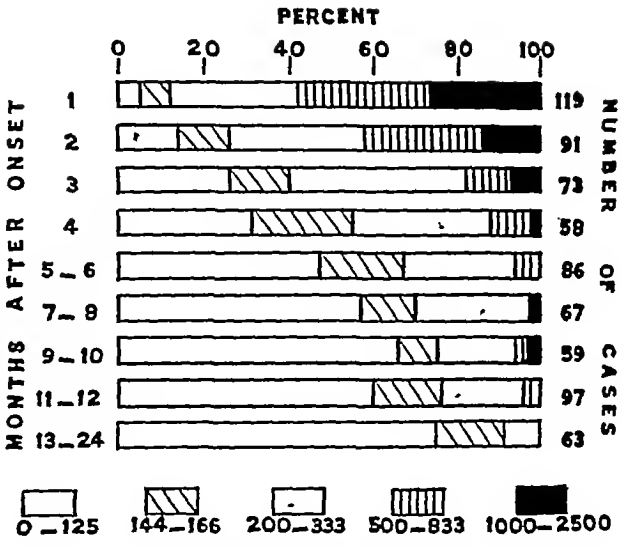


FIG 2 TITER OF ANTISTREPTOLYSIN FOLLOWING ACUTE GLOMERULONEPHRITIS

of the patients are normal and 25 per cent have titers from 144 to 333 units At this time the nephritis had healed in all but 8 patients This period serves as a control for the group and these figures are in good agreement with the control group of Coburn and Pauli in New York City who found that 75 per cent of 146 individuals had titers of 100 units or below

In Figure 3 are presented curves to show the variations in antistreptolysin titer in the first year after the onset of nephritis In 71 patients determinations were made frequently enough so that a significant curve could be made. These curves fall roughly into 7 groups, two curves are shown as representative of each group

Group A Thirteen of the 71 patients showed this type of response Five of the 8 patients who developed chronic nephritis were in this group The significance of this will be discussed later

Group B Thirteen patients showed this type of curve, *i e*, a moderate initial rise and a prompt fall to normal levels All except one individual recovered completely

Group C Ten patients showed this type of curve, an initial rise to 500 units with a tendency to moderate elevation of titer at the end of the first year All these patients recovered

Group D Twelve patients showed this type of response, a high initial rise (800 units) with normal titers by the third month Two patients who

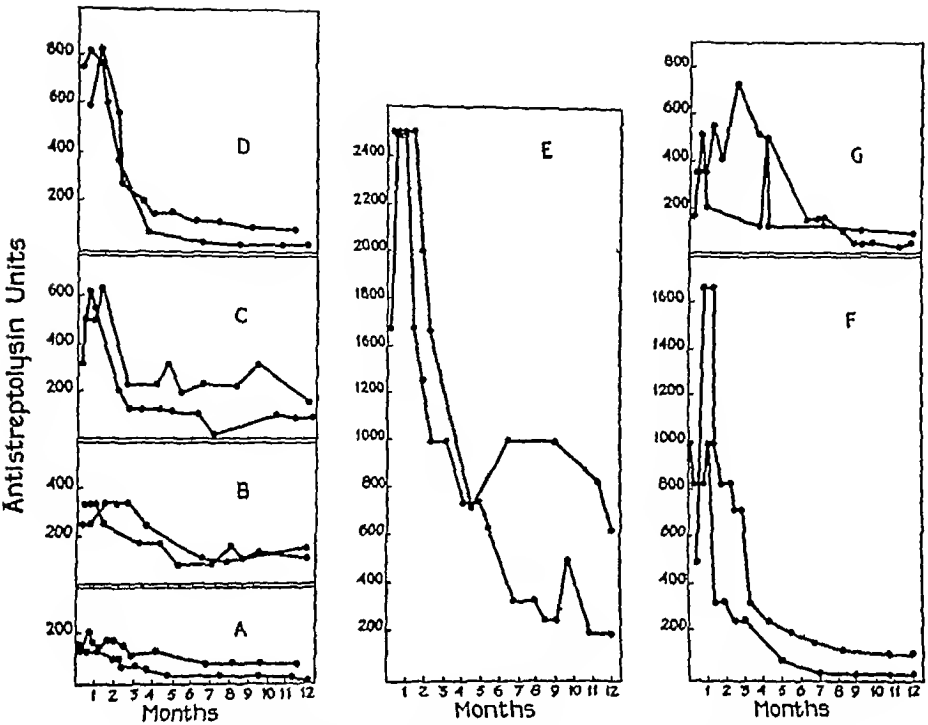


FIG. 3 VARIATIONS IN ANTISTREPTOLYSIN TITER IN THE FIRST YEAR OF ACUTE NEPHRITIS

developed chronic nephritis showed this type of response.

Group E Three patients showed extremely high initial titers with a slow fall toward normal levels but at the end of the first year the titers were still abnormally high

Group F Thirteen patients showed extremely high initial titers with normal titers after the 6th month. All recovered

Group G Seven patients showed either a rising titer in convalescence or a secondary rise after the initial rise and fall in titers In all these patients there was an exacerbation of the infection or a reinfection by hemolytic streptococcus

DISCUSSION

The great majority of individuals who have hemolytic streptococcal infection followed by an attack of acute glomerulonephritis develop a significant rise in antistreptolysin titer But this response is known to occur in individuals in whom hemolytic streptococcal infection is not followed by acute glomerulonephritis

The maximum antistreptolysin titer is present at the onset or during the first few weeks of the attack of acute nephritis but the significance of this fact is not apparent The maximum rise in antistreptolysin titer may be of all degrees and the clinical findings in the patients who developed extremely high antistreptolysin titers are not different from those who developed a moderate or intermediate rise in antistreptolysin titer

The fact that 5 of the 8 patients who developed chronic nephritis showed only moderate elevation of antistreptolysin titer (Group A, Figure 3) is interesting

The value of x^2 calculated from Table IV using Yates correction is 8 676 The value re-

TABLE IV

Relation of antistreptolysin titer and the development of chronic nephritis

	Moderate elevation of antistreptolysin titer	Intermediate and high elevation of antistreptolysin titer	Total
Chronic nephritis	5	3	8
Healed nephritis	8	55	63
Total	13	58	71

quired to demonstrate significant ($P = 0.01$) deviation from random sampling = 6.635. While these figures are statistically significant there are too many other factors to be considered in the evolution of chronic glomerulonephritis and the number of cases is too small to make possible a definite conclusion. Further study may throw more light on this point.

In the majority of patients the antistreptolysin titer begins to fall in the first or second month of the disease and in half the patients the antistreptolysin titer is normal by the 6th month. In this period the nephritis begins to improve clinically as measured by the disappearance of hypertension and edema, diminution in albuminuria and hematuria, and by the return of normal kidney function. But this finding may be coincidental rather than correlative since analysis of the data accumulated in the 2-year period after the onset of the nephritis shows no correlation between the rate at which the antistreptolysin titer returns to normal and the clinical changes in the patient. In the second year after the onset of the nephritis when 108 of the 116 patients were healed, the antistreptolysin titer was normal in 75 per cent with 25 per cent of the patients showing a moderate elevation (144 to 333 units).

It is well known that antibody responses to various infections may persist for a long time after the acute infection has subsided. That this is true also of antistreptolysin is shown in the charts. Recently Todd expressed the opinion based on animal work that the persistence of high antistreptolysin titers in convalescence signifies persistence of the infection. A study of our cases indicates that this is probably true in hemolytic streptococcal infection in man. In all 31 of our patients in whom high titers either persisted or recurred after the initial rise and fall there was definite clinical or bacteriological evidence of the persistence or recurrence of infection. These patients were carefully studied for information as to the effect on the nephritis of the persistent or recurrent infection. In only 10 of the 31 patients could it be said that the infection had an adverse effect on the nephritis. This effect was shown in some cases by an increase in hematuria and albuminuria and in others by the persistence of well-marked hematuria and albuminuria. It is believed by some workers that persistence of the infection or the recurrence of infection in convalescence from nephritis is associated with persistence or exacerbation of the nephritis. This may be true in some cases but it is certainly not the rule. Longcope states that when "the disease progressed to a chronic stage the antistreptolysin titer remained at a high level in a fair proportion of instances." The rôle of hemolytic streptococcal infection on the course of chronic nephritis is to be considered elsewhere (12) but it can be said here that, in this series, in the 8 patients who progressed to chronic nephritis the level of antistreptolysin was normal in all at 6 months and has remained normal during the subsequent period of observation.

CONCLUSIONS

1. In 116 consecutive cases of acute glomerulonephritis the bacteriological data indicate that 71.5 per cent had a prodromal hemolytic streptococcal infection, and the immunological data show that 94 per cent had had a recent hemolytic streptococcal infection.

2. The height and duration of the antistreptolysin titer in patients with acute glomerulonephritis appear to be related to the severity, persistence, or recurrence of the hemolytic streptococcal infection.

3. Analysis of the immunological and clinical data in the present study does not show any significant correlation between the height and duration of the antistreptolysin response and the severity or duration of the acute attack of nephritis or the tendency to develop chronic nephritis.

4. There is a wide variation in the curve of antistreptolysin response constructed over a long period of time. The form of the curves has no relation to the severity or duration of acute ne-

phritis or to the tendency to develop chronic nephritis

BIBLIOGRAPHY

1 Seegal, D., and Lyttle, John D Antistreptolysm titre of the serum in acute glomerular nephritis. Proc. Soc. Exper Biol. and Med., 1933 31 211.

2. Todd E. W., Antigenic streptococcal hemolysm. J Exper Med., 1932, 55 267

Todd, E. W., Antihaemolysm titres in haemolytic streptococcal infections and their significance in rheumatic fever Brit. J Exper Path., 1932, 13, 248

3 Coburn, A F., and Pauli, Ruth H., Studies on the immune response of the rheumatic subject and its relationship to activity of the rheumatic process VI. The significance of the rise of antistreptolysin level in the development of rheumatic activity J Clin Invest., 1935 14, 769

4 Griffiths G J., Antihaemolysin titres in chronic rheumatic and allied diseases. Lancet, 1934 2, 251

5 Myers, W K., and Keefer C. S., Antistreptolysio content of the blood serum in rheumatic fever and rheumatoid arthritis J Clin. Invest., 1934, 13, 155

6 Lippard, V W., and Johnson, P., Beta hemolytic streptococcic infection in infancy and in childhood. I Antifibrinolysin and antistreptolysin response. Am. J Dis Child., 1935 49, 1411

7 Wilson, May G, Wheeler, G W., and Leask, M M., The relation of upper respiratory infections to rheumatic fever in children. II. Antihemolysio titres in respiratory infections and their signifi cance in rheumatic fever in children. J Clin. Invest., 1935, 14, 333

8 Blair, J E., and Hallman, F A. Streptococcal ag glutinins and antistreptolysins in rheumatoid (atrophic) arthritis J Clin. Invest., 1935, 14, 505

9 Longcope, W T., Studies of the variations in the antistreptolysin titer of the blood serum from patients with hemorrhagic nephritis I Control observations on healthy individuals and patients suffering from diseases other than streptococcal infections. J Clin. Invest., 1936 15, 269

II Observations on patients suffering from streptococcal infections, rheumatic fever and acute and chronic hemorrhagic nephritis. J Clin. In vest., 1936, 15, 277

10 Todd, E. W., and Hewitt, L. F., A new culture me dium for the production of antigenic streptococcal hemolysin. J Path. and Bact., 1932, 35, 973

11 Hodge, B E., and Swift, H. F., Varying hemolytic and constant combining capacity of streptolysins influence on testing for antistreptolysins J Exper Med., 1933 58, 277

12. Seegal D., Lyttle, J D Loeb, E. N., and Jost, E. L. The rôle of hemolytic streptococcal infection in chronic glomerulonephritis (Unpublished obser vations)

EXPERIENCE WITH THE HAMILTON AND HIGHMAN TEST FOR PARATHYROID HYPERFUNCTION IN CHRONIC NEPHRITIS, TOXIC GOITER, AND PAGET'S DISEASE OF BONE

By D ROURKE GILLIGAN, MARIE C. VOLK, AND S L. GARGILL

(From the Medical Research Laboratories of the Beth Israel Hospital and the Department of Medicine Harvard Medical School Boston)

(Received for publication April 29 1938)

In 1932 Hamilton and Schwartz (1) described a method for the detection of small amounts of parathyroid hormone, three to five units, in preparations of the hormone and in blood In 1936 Hamilton and Highman (2) presented certain modifications of this test designed to make the method particularly applicable to the detection of abnormally large amounts of parathyroid hormone in the blood of patients suspected of having increased parathyroid function The test consists, briefly, in measuring the increase in the serum calcium of a rabbit at definite intervals after the rabbit has received an intramuscular injection of the blood or preparation containing parathyroid hormone, the animal being given amounts of calcium chloride solution by stomach tube at definite periods during the experiment

In this communication we present our results obtained with the Hamilton and Highman test, and related blood chemical studies, in patients with chronic nephritis The parathyroid glands of some of the patients were examined postmortem Results obtained in toxic goiter and Paget s disease of bone are also briefly presented and discussed

REVIEW OF LITERATURE

Io the past six years Hamilton and his coworkers have applied this test to studies of the amounts of parathyroid hormone in the blood of experimental animals and of patients in whom hyperfunction of the parathyroid glands was suspected. These aothors performed the test on the blood of 38 normal indvidoals in these experiments the greatest rise In rabbits serum calcium obtained in any experiment at either the three hour or five-hour period after injection of the blood into the rabbits was 0.23 mM per liter (2) These findings in normal individuals are used as a control basis the fioding of an increase of 0.30 mM or more in the serum calcium of a rabbit is taken to indicate abnormally increased parathormone in the blood of a patient under study the test is not applicable to the measurement of abnormally small amounts of circulating parathyroid hormone (2)

Hamilton and Schwartz (3) found evidences of increased parathormone in the blood, according to their test, in 9 of 12 rachitic rabbits. In this same communication the aothors also reported that the blood calcium of the rachitic rabbits increased immediately and markedly when calcium chloride was administered to the animals by stomach tube whereas there was a much more moderate rise in the calcium coocentration in a series of normal rabbits so tested the aothors have since reported that these results could not be reproduced in other series of rachitic rabbits (4) Io one patient with intractable rickets Highman and Hamiltoo found a positive test on two occasions (5), Kajdi and Shelling found a positive test in a case of florid rickets of several years duration (6)

In a study of 74 pregnant women Hamilton, Dasef Highman and Schwartz found evidences of increased *parathyroid hormone in the blood of 60 per cent of the* cases studied between the fifth and seventh months of pregnancy (7) Only 3 of 13 women in the last two months of pregnancy showed positive tests only 1 of 11 lactating women showed a positive test. This apparent increase in circulating parathyroid hormone during pregnancy accords with histological findings indicative of parathyroid hyperactivity (8, 9) and with the findings of Hoffmann *et al* (10 11) who demonstrated the presence in blood from pregnant women of a substance which behaves like parathyroid hormone. Hamilton *et al* (7) point out that whereas increased parathyroid hormone was only occasionally found by their test in the blood of women in the tenth month of pregnancy Hoffmann (10) found in this period the greatest amount of substance which acted like parathyroid hormone.

Highman and Hamilton found abnormally great amounts of parathyroid hormone in the blood of 20 of 23 patients with chronic nephritis and elevated blood urea nitrogen (12) Shelling and Remsen (13) reported a case of renal rickets with elevated concentrations of nitrogen and inorganic phosphorus in the blood which case showed increased parathyroid hormone in the blood according to the Hamilton and Schwartz test, and at postmortem examination showed four markedly enlarged parathyroid glandules These authors mention that the results of the Hamilton and Schwartz test were negative in other cases of renal rickets. Bass and Paxter (38) recently performed the "Hamilton test" on two occasions in one patient with renal rickets on one of these occasions the result was suggestlvely positive," on the other trial, negative.

The present authors (14), in a preliminary communication, reported that 4 of 6 patients with thyrotoxicosis showed increased parathyroid hormone in the blood, according to the criteria of Hamilton and Highman.

Kajdi and Shelling (6) performed the Hamilton and Schwartz test in patients suffering from various skeletal diseases including Paget's disease, xanthomatosis ossium, the cases of renal rickets and the one with rickets mentioned above, and a case of osteitis fibrosa The case of osteitis fibrosa, the one with rickets, and one of those with renal rickets showed increased blood parathyroid hormone, the other cases did not.

Hamilton and Schwartz have found a considerable variation in the magnitude of the serum calcium increase in different rabbits after a given amount of parathyroid has been injected (1) In the animals into which 1 5 or more units of parathormone per kilo were injected, however, the highest serum calcium values attained, either at the three-hour or five-hour period, were always greater than any occurring in control animals to which calcium chloride was given by stomach tube but to which no parathormone was administered Dyer (15) has attempted to apply the test of Hamilton and Schwartz to the standardization of parathyroid extracts but concluded that the method was useful in detecting but not in quantitating small amounts of parathyroid hormone

METHODS

Blood, both for the rabbit test and chemical studies, was drawn from patients who had fasted overnight in all instances in which blood chemical measurements were made.

The Hamilton and Highman test (2) for increased parathyroid hormone in the blood was performed exactly as described by the authors A rise of 0.30 mM per liter, or greater, in the calcium of the serum of a test rabbit either at the three-hour or five-hour period was considered a "positive" reaction Certain details of the method were discussed with Dr Hamilton through his kind cooperation in personal communication. Rabbits weighing from 1 5 to 4 kilos were utilized, most of the animals weighed from 2 5 to 3 5 kilos No rabbit was utilized for a second test until at least three weeks after a previous test, whether the result had been positive or negative. The rabbits were fed Purina Rabbit Chow for several days or weeks before being used for the test. In 82 per cent of the animals the control calcium concentrations of the sera were from 2 8 to 3 4 (inclusive) mM per liter, the extreme limits for all animals being 2 4 mM and 3.5 mM per liter In approximately 75 per cent of the experiments, sufficient blood was drawn from the rabbits to make duplicate measurements of the serum calcium on all three occasions of sampling, *i e*, at the control, three-hour, and five-hour periods It was noted rather frequently that the withdrawal of 10 cc. of blood on three successive occasions caused a considerable lowering of the hematocrit, it was presumed that lowering of serum protein also occurred and that this effect might occasionally mask a rise in the diffusible serum calcium It was learned by personal communication from Dr Hamilton that in his laboratory only sufficient blood for a single measurement of calcium was drawn at each period. We repeated the test in this manner in several patients in whom results had been negative previously when blood samples large enough for duplicate calcium measurements had been drawn, in each instance negative results were also obtained on these repeat trials

The calcium concentrations of the sera of the rabbits and of the patients were measured according to Fiske and Logan (16), inorganic phosphorus concentrations of the sera according to Fiske and Subbarow (17) Plasma phosphatase measurements were made by the original method of Kay (18), by which method the upper limit of normal values in adults is 0.21 units Nonprotein nitrogen was measured on the trichloracetic acid filtrate of the serum by the micro-Kjeldahl method Total protein was measured by the macro-Kjeldahl method (19)

RESULTS

Normal individuals

The Hamilton and Highman test was performed in 5 instances in 4 normal adult subjects The maximum increase in calcium of any of the test rabbits was 0 19 mM per liter (Table I) The

TABLE I

Results of the Hamilton and Highman test in normal adults

Case	Sex	Age	H and H * test
		years	*mM per liter*
1 D G	F	35	−0 26
	F	35	+0 19
2 M V	F	27	+0 19
3 W M	M	25	+0 08
4 G L	M	24	+0 08

* "H and H" refers to Hamilton and Highman in this table and in Table II, the figures in this column refer to the maximum rise in the serum calcium of the rabbit

results in this small series accord with those found in 38 normal individuals by the authors of the method

Chronic renal insufficiency

The test was performed in 19 instances in 15 patients with chronic renal disease (Table II) With the exception of 4 cases (Cases 4, 12, 13, and 14) the patients were hospitalized because of uremic symptoms, including drowsiness, nausea, vomiting, muscular twitchings and cramps, and purpuric manifestations Most of these hospital-

TABLE II

Results obtained with the Hamilton and Highman test in patients with nephritis together with pertinent blood chemical findings

Case	Sex	Age	Diagnosis	Blood pressure	Serum chemical findings				Plasma phosphatase	H and H test
					Calcium	Phosphorus	Protein	Non protein nitrogen		
		years		*mm Hg*	*mgm per 100 cc.*	*mgm per 100 cc*	*grams per 100 cc.*	*mgm per 100 cc.*	*Kay units*	*mM per liter*
1 D C.	M	35	Uremia	130/80	6 7	8 6	6 0	126	0 11	+0 07
2 B T	M	22	Uremia	160/80	8 4	7 0	5 8	96		−0 08
B T	M	22	Uremia		8 4	5.3		122		−0 06
3 R. M	F	52	Uremia	150/90	5 0	13 1	6.5	263	0 19	−0 20
4 R S	F	44	Uremia	160/100	5 0	8 8	5.3	211	0 14	+0 07
5 J P	M	34	Uremia	190/110	5 6	10 9	7 1	271		−0 11
6 E. S	M	34	Uremia	160/90	6.3	11 1	7 7	213	0 10	+0 08
7 J Mc	M	45	Uremia	150/95	6.3	9 9	7 0	107	0 17	+0 10
8 B M	F	40	Uremia	142/84	6 4	10 4	5.5	152	0 19	+0 47
9 L. M	M	58	Uremia	180/80	6 4	9 7	6.3	124	0.31	+0 17
10 A. F	F	40	Uremia	170/90	3 9	9 5	5.3	139	0 19	+0 01
11 S C.	F	18	Uremia	170/120	4 0	9 1	5 1	195		+0 22
S C	F	18	Uremia		6 4	8 0	5 8	131		−0 06
12 A. W	M	15	Renal rickets	120/60	8.5	7 0	6 2	126	2 01	−0 11
A. W	M	15	Renal rickets							−0 18
A. W	M	15	Renal rickets	110/70	8 5	5 8	6 7	97	1 91	+0 26
13 L. H.	F	53	Chronic nephritis	240/130	8 8	4 3	6.5	79		−0 25
14 M C.	M	66	Chronic nephritis	170/90	9 2	4.3	7 7	64		+0 08
15 B B	F	10	Renal rickets	120/70	10 0	7 6		113	27*	−0 20

* Bodansky units

ized cases died within a few weeks of the time of our studies Usual therapeutic procedures were employed in these cases

The nonprotein nitrogen of the serum was elevated in every case, the inorganic phosphorus was elevated in all but two cases (Cases 13 and 14) The protein of the serum was less than 6.5 grams per cent in 8 cases The calcium of the serum of all cases was between 3.9 and 10 0 mgm per 100 cc., the highest calcium value of 10 0 mgm. was found in a case with renal rickets (Case 15) [1] In 10 of the 15 patients the serum calcium was less than 7 0 mgm per 100 cc , the two very lowest concentrations of calcium were found in patients with the lowest concentrations of serum protein The phosphatase of the plasma was markedly elevated in the two patients with renal rickets (Cases 12 and 15), in one other case a somewhat elevated value was found (Case 9)

In 18 of the 19 experiments the Hamilton and Highman test was negative, the maximum rises in the calcium of the rabbits sera at the three-hour or five hour periods being not greater than 0.30 mM per liter In three of these cases showing negative results the test was repeated on one or more occasions, the repeat tests were likewise negative. In one patient (Case 8) the test was positive, the rabbit calcium increasing by 0 47 mM above the control at both the three-hour and five-hour periods

The parathyroid glands were examined post-mortem in 4 of the patients who died in uremia (Table III), one of these cases had renal rickets (Case 12) In each instance the glands showed enlargement and hyperplasia of the "secondary" type (23) (Table III), the greatest hypertrophy being found in the patient with renal rickets In all of these cases the parathyroid function test, performed from 1 to 4 months before death, was negative. In 2 of the 4 cases the concentrations of serum calcium were below 7 0 mgm per 100 cc.

Other clinical conditions

During our investigations in patients with nephritis, we have also accumulated data on the results of the Hamilton and Highman test in a series of patients with Paget's disease, and a series with thyrotoxicosis

The test was performed in 8 patients with *ac-*

[1] This case was kindly referred to us by Dr A. M Butler of the Children s Hospital, Boston.

TABLE III

Parathyroid morphology in patients with chronic nephritis

Case (as of Table II)	Weight of glands				Combined weight of glands	Measurements of glands			
	Left lower	Left upper	Right lower	Right upper		Left lower	Left upper	Right lower	Right upper
	mgm	*mgm*	*mgm*	*mgm*	*mgm*	*mm*	*mm*	*mm*	*mm*
1 D C.	113	21	150	76 21	421	6×7×3	9×5×2	8×6×3	9×4×2 3×2×1
2 B T	37	35	73	74	269	5×6×3	11×6×3	10×5×2	9×4×2
11 S C	34	35	37	36	142	4×3×2	4×3×2	4×3×2	4×3×2
12 A W *	170	1.2	157	90	549	11×7×5	10×4×2	11×6×4	10×4×2

Microscopic findings

Case 1 Cells in dense cords and masses, with tendency toward papillomatous and adenomatous arrangement in many areas Slightly enlarged chief cells predominant, many showing considerable halo formation Occasional greatly enlarged chief cells seen Oxyphilic cells about normal in number, fat decreased Left lower gland showed alveolus formation

Case 2 Cells in dense cords and masses, with tendency toward papillomatous and adenomatous arrangement in many areas Slightly enlarged chief cells predominant, many showing considerable halo formation Oxyphilic cells increased in number, fat decreased

Case 11 Findings same as in Case 2, except for slight increase in fibrous tissue in one gland

Case 12 * Findings as in Case 2

* Renal rickets

tive Paget's disease All of the cases showed clinical and roentgen ray findings typical of the disease, and markedly elevated plasma phosphatase values, all had more than one bone involved by the disease, most of them showing "generalized Paget's disease" The serum calcium, phosphorus, and protein concentrations were essentially normal The Hamilton and Highman test was positive in 3 of the 8 cases, the rises in the calcium of the sera of the test rabbits in these instances being greater than 0 30 mM In two cases in which the results were negative, the tests were repeated and again found negative

The test was found positive in 7 of 18 patients with thyrotoxicosis Of the first 7 patients studied, 5 showed a positive test, whereas in the last 11 cases only two tests were positive In one case showing a positive result the test was repeated and again found positive Two patients who showed positive results when thyrotoxic showed negative results after treatment with iodine and operation The cases studied showed varying degrees of toxicity, some had received iodine for one to three days before the test was made We have not discovered any characteristic differences in the clinical or laboratory findings between the patients who showed positive and those who showed negative tests In 16 cases the bony calcification of the hands was compared with that of normal subjects of approximately the same ages by taking roentgenograms of both simultaneously Slight osteoporosis was observed in this manner in one case, the parathyroid function test in this patient was positive The plasma phosphatase was slightly increased in several patients, the result of the parathyroid function test was not related to the phosphatase values Normal values for concentrations of calcium, phosphorus, and protein in the sera were found

DISCUSSION

Enlargement of the parathyroid glands has been shown to occur frequently in patients with chronic renal insufficiency (20, 21, 22, 23) Among the most striking cases of this enlargement are those occurring in patients with renal rickets in whom chronic renal insufficiency is of a marked degree and of long duration (13, 24, 25, 26, 27) This enlargement has recently been shown to be due primarily to a chief cell or "secondary" hyperplasia (13, 20, 23, 26, 27), this hyperplasia, at times without apparent gross enlargement of the glands, occurs with regularity in all cases with chronic renal insufficiency of long duration (23) Enlargement of the parathyroids and "secondary" hyperplasia has been found in other clinical

conditions such as rickets and osteomalacia (28, 29, 30, 31) and occasionally in Paget's disease (23, 32, 33)

Whereas all cases with parathyroid tumors and accompanying parathyroid hyperfunction, and cases with the "primary" or Wasserhelle type of hyperplasia probably show, eventually, characteristic blood chemical, clinical, and x-ray changes, osteitis fibrosa and increased serum calcium are encountered relatively with great rarity in patients with the "secondary" type of hyperplasia (23) Castleman and Mallory (23) feel that the occasional development of osteitis fibrosa in patients with renal insufficiency depends upon a long duration of the disease.

The chief purpose of the Hamilton and Highman test is to discover the presence or absence of parathyroid hyperfunction in cases which are suspected of having hyperfunction due to "secondary" hyperplasia The test would, if reliable, clarify the relationship between anatomical findings in such cases, and function

Our original purpose in studying the parathyroid function test in patients with chronic renal insufficiency was to compare, in the same subject, the results of the test with the degree of enlargement and hyperplasia of the parathyroid glands found at postmortem examination, in those cases in which autopsies could be made. It was thought that the meaning of the test would be clarified through such studies These thoughts were formulated and some of our results obtained before publication of Highman and Hamilton's communication concerning the parathyroid function test in nephritis (12)

The negative results obtained with the test in 14 of our 15 patients with chronic renal insufficiency (Table II) are quite at variance with the positive results found by Highman and Hamilton in 20 of their series of 23 patients In four of our cases showing no increased function by the test we were able to examine the parathyroid glands postmortem and found slight to marked enlargement, and hyperplasia of the "secondary" type in every case, the greatest enlargement was observed in a case of renal rickets (Case 12) (Table III) Presumably, varying degrees of parathyroid enlargement and hyperplasia were present in the other cases of our study as well As was reported above, Kajdi and Shelling (6) found indications of increased parathyroid function by the Hamilton and Schwartz test (1) in one case with renal rickets and marked enlargement of the parathyroid glands However, these authors (6), and Bass and Paxter (38) found negative results in other cases of renal rickets who presumably also had parathyroid hyperplasia. Two of the cases of our series had renal rickets, and both showed negative results with the Hamilton and Highman test, postmortem examination in one of these cases revealed marked enlargement, with "secondary" hyperplasia, of the parathyroids

Our series of nephritic cases accords with that of Highman and Hamilton (12) in that the patients had chronic nephritis and elevation of blood urea nitrogen Most of our cases showed phosphorus retention and somewhat lowered plasma protein concentration, this is likewise true of Highman's series

The concentrations of calcium in the sera of our nephritic cases were notably lower than those reported by Highman and Hamilton The latter authors reported a serum calcium concentration below 8 mgm per 100 cc. in only one of their series of 23 cases, whereas 10 of our 15 cases showed marked reduction in serum calcium concentration Low calcium values have usually been found by others in patients with chronic nephritis and marked nitrogen and phosphorus retention (23, 34, 35, 36) Low serum calcium and enlargement of the parathyroids with "secondary" hyperplasia may, and usually do, coexist in chronic renal insufficiency, as shown by cases in our series and in those of Castleman and Mallory (23) It is worthy of note, in this connection, that in many of the reported cases of renal rickets, as well as in one of our cases (Case 15) with this condition, serum calcium values of 9 5 to 13 mgm per 100 cc. have been found, in the presence of high serum concentrations of inorganic phosphorus and nitrogen (13, 26, 27, 38) As mentioned above, very marked degrees of "secondary" hyperplasia of the parathyroids are found in patients with this syndrome (13, 24, 25, 26 27)

It would appear from this discussion that in chronic renal insufficiency the results obtained with the Hamilton and Highman test are not reproducible in the hands of different investigators

It is also true that in our h[illegible] the test does not indicate increased parathyroid hormone in the blood of certain patients who have enlarged parathyroid glands showing the "secondary" type of hyperplasia

That Hamilton and Schwartz were unable to reproduce their original results (4) showing marked increase in the serum calcium concentration of rachitic rabbits given soluble calcium salts by stomach tube, is disturbing, because of the similarity of the theory and the practice of this procedure with that utilized in the parathyroid function test This finding suggests either some peculiarity of technique which was not reproduced in their second trial and in that of McCoy (see reference (4)), or the inconstancy of the response of different series of test animals (4) That the increases of serum calcium in individual rabbits differ considerably after a given dose of parathyroid hormone under the conditions of the Hamilton and Schwartz parathyroid function test has been recognized by Hamilton and Schwartz (1) and by Dyer (15), that large series of rachitic rabbits should vary inherently so importantly as to show consistent responses to calcium salts by stomach tube in individual animals within a series but quite opposite responses in other series is not understandable

We have had no experience with the test in simple rickets or in pregnancy As noted above we have found positive results in a certain percentage of cases with Paget's disease and cases with thyrotoxicosis Our investigation in Paget's disease was prompted by the reported findings of enlargement and "secondary" hyperplasia of the parathyroid glands in certain individuals with this disease (23, 32, 33), the investigation in thyrotoxicosis was undertaken on the suspicion that the parathyroids might become hyperactive in the presence of hyperthyroidism, and because of the osteoporosis occasionally observed in patients with thyrotoxicosis (37) Kajdi and Shelling (6) have reported a positive result in a patient with osteitis fibrosa cystica, they found negative results in a few cases with Paget's disease

On the basis of present knowledge we feel that the significance of the test is not clear, it cannot be stated, therefore, whether those cases in whom we obtained positive results had a greater degree of functional hyperparathyroidism than those in whom negative results were found

SUMMARY

1 Parathyroid hyperfunction was not indicated by the Hamilton and Highman test in 13 of 14 cases with chronic renal insufficiency

These findings do not accord with the large percentage of positive results found with the same test in a similar series of nephritic patients by the authors of the test

2 In 4 of the nephritic cases of this study with negative results, the parathyroid glands were found, at postmortem examination, to be enlarged and to show "secondary" hyperplasia One of these patients had renal rickets

3 It is pointed out that the serum calcium values reported by Hamilton and Highman in their series are notably higher than those found by us and others in most patients with advanced renal insufficiency and phosphorus retention

4 The parathyroid function test was found positive in some patients with thyrotoxicosis and some with Paget's disease

5 Because of the conflicting results obtained with the method by different investigators the significance of the test is, at present, questionable

We are indebted to Dr Mark D Altschule for dissection and histological examination of the parathyroid glands described in this communication

BIBLIOGRAPHY

1 Hamilton, B, and Schwartz, C., A method for the determination of small amounts of parathyroid hormone. J Pharmacol and Exper Therap, 1932, 46, 285

2 Hamilton, B, and Highman, W J, Jr., A test for abnormally large amounts of parathyroid hormone in the blood. J Clin. Invest., 1936, 15, 99

3 Hamilton, B, and Schwartz, C, Rickets and hyperparathyroidism Am. J Dis Child., 1933, 46, 775

4 Hamilton, B, Absorption of ingested calcium in rachitic rabbits Am J Dis Child (Correspondence), 1938, 55, 160

5 Highman, W J, Jr, and Hamilton, B, Calcium and phosphorus metabolism in a case of intractable rickets J Pediat., 1936, 9, 56

6 Kajdi, L., and Shelling, D H, see Reference (13)

7 Hamilton, B, Dasef, Laura, Highman, W J, Jr, and Schwartz, C., Parathyroid hormone in the blood of pregnant women J Clin Invest, 1936, 15, 323

8 Seitz, L., Eklampsie und parathyreoidea. Arch f Gynäk., 1909, 89, 53
9 Marine, D., The Thyroid, Parathyroids and Thymus. See Cowdry, E. V., Special Cytology Vol II Paul B Hoeber, Inc. New York, 1932, 2d ed., p. 831
10 Hoffmann, F., Über die Darstellung und den Nachweis des Nebenschilddrüsenhormons im Schwangerenblut. Arch. f Gynak., 1933, 153, 181
11 Hoffmann, F, and Rhoden, E., Untersuchungen uber die Wirkung des Nebenschilddrüsenhormons aus Schwangerenblut. Arch. f Gynäk., 1934, 156, 459
12. Highman, W J., Jr., and Hamilton, B Hyperparathyroidism in kidney disease. J Clin. Invest., 1937, 16, 103
13 Shelling D H., and Remsen, D Renal rickets. Report of a case showing four enlarged parathyroids and evidence of parathyroid hypersecretion Bull Johns Hopkins Hosp, 1935 57, 158
14 Gilligan, D R., Gargill, S L., and Volk, M. C., Parathyroid function in hyperthyroidism. Proc. Soc. Exper Biol. and Med., 1936 35, 89
15 Dyer F J., The use of rabbits in the standardisation of parathyroid hormone. Quart. J Pharm. and Pharmacol., 1935, 8, 197
16 Fiske, C. H., and Logan M A., The determination of calcium by alkalimetric titration. II The precipitation of calcium in the presence of magnesium, phosphate and sulfate, with applications to the analysis of urine. J Biol. Chem., 1931 93 211
17 Fiske, C. H and Subbarow, Y., The colorimetric determination of phosphorus J Biol. Chem., 1925, 66, 375
18. Kay H. D, Plasma phosphatase. I Method of determination. Some properties of the enzyme. J Biol. Chem., 1930 89, 235
19 Dyer, B., Kjeldahl's method for the determination of nitrogen. J Chem. Soc. Trans., 1895, 67 811
20 Bergstrand, H., Parathyreoideastudien. II Über Tumoren und hyperplastische Zustande der Nebenschilddrusen Acta med. Scandinav 1921 54, 539
21 Pappenheimer, A. M and Wilens, S L. Enlargement of the parathyroid glands in renal disease. Am. J Path., 1935 11, 73
22. Gilmour, J R., and Martin, W J, The weight of the parathyroid glands. J Path. and Bact., 1937 44, 431
23 Castleman B, and Mallory T B., Parathyroid hyperplasia in chronic renal insufficiency Am. J Path., 1937, 13, 553
24 Vogt, E. C., Renal rickets Am. J Roentgenol., 1933 30, 624
25 Langmead, F S., and Orr, J W., Renal rickets associated with parathyroid hyperplasia. Arch. Dis. Childhood, 1933, 8, 265
26 Smyth, F S, and Goldman, L., Renal rickets with metastatic calcification and parathyroid dysfunction. Am. J Dis. Child., 1934, 48, 596
27 Price, N L., and Davie, T B., Renal rickets. Brit. J Surg., 1937, 24, 548
28. Erdheim, J., Quoted by Biedl, A. Innere Sekretion. Part 1 Urban and Schwarzenberg, Berlin, 1913, 2d ed., p. 108.
29 Pappenheimer A. M, and Minor, J., Hyperplasia of the parathyroids in human rickets. J Medical Research, 1921, 42, 391
30 Hoffheinz, Über Vergrösserungen der Epithelkörperchen bei Ostitis fibrosa und verwandten Krankheitsbildern. Virchows Arch. f path. Anat., 1925, 256 705
31 Ritter, C., Über Epithelkörperchenbefunde bei Rachitis und anderen Knochenerkrankungen. Frankfurt Ztschr f Path., 1920 24, 137
32. Ballin, M., Parathyroidism. Ann. Surg., 1932, 96, 649
33 Shambaugh, P., and Cutler, E. C., Survival of an autoplastic parathyroid transplant in a patient without parathyroid deficiency Arch Surg., 1936 32, 842.
34 de Wesselow, O L. V., On the phosphorus and calcium of the blood in renal disease. Quart. J Med., 1923 16, 341
35 Schmitz, H. W, Rohdenburg E. L., and Myers, V C., The inorganic phosphorus and calcium of the blood in nephritis. Arch. Int. Med., 1926, 37, 233
36. Peters, J P., and Eisenson, L., The influence of protein and inorganic phosphorus on serum calcium. J Biol. Chem., 1929, 84, 155
37 Aub, J C., Baner, W Heath, C., and Ropes, Marion Studies of calcium and phosphorus metabolism. III The effects of the thyroid hormone and thyroid disease. J Clin. Invest., 1929 7, 97
38. Bass M. H., and Paxter, Jean, Congenital polycystic kidneys with secondary bone changes (renal hyperparathyroidism, renal rickets) J Mt. Sinai Hosp. 1938 4 882.

THE CHOLINE-ESTERASE ACTIVITY OF THE BLOOD SERUM IN DISEASE

By A. T MILHORAT

(From the New York Hospital and Departments of Medicine and Pharmacology Cornell University Medical College and the Russell Sage Institute of Pathology New York City)

(Received for publication May 9 1938)

The investigations of Dale and his coworkers (1, 2) on the chemical transmission of parasympathetic nerve stimulation and of Walker (3, 4) on the use of physostigmine and prostigmin in myasthenia gravis have stimulated interest in the choline-esterase activity of serum. Lucas, Hall, and Ettinger (5) studied the serum esterase activity of 200 subjects and obtained results that were in essential agreement with the earlier findings of Stedman, Stedman, and Easson (6) and the more recent ones of McGeorge (7) on 132 patients Although wide variations occurred among the subjects, the esterase activity was found to be constant over periods of many weeks, and no correlation with any type of clinical syndrome could be found However, in myasthenia gravis conflicting findings have been reported. Thus, Hicks (8) found in one patient an abnormally high esterase activity which increased even further during exacerbations of the muscular symptoms Stedman and Russell (9), on the other hand, observed serum esterase values in myasthenia gravis that were lower than in the other clinical conditions they studied, and believed that the distribution of the enzyme between the corpuscles and the serum was different from that in other conditions

In the present investigations the choline esterase of the sera of 109 subjects was determined The clinical syndromes studied included a wide variety of conditions Some of the subjects were without organic disease, many were only moderately ill, and others were in the advanced or even final stages of disease. In many instances the serum esterase activity was studied at frequent intervals over periods of several months The effect of many factors was investigated These included the age, sex, body weight, and total muscular mass of the subjects changes in the clinical status, convulsions, fasting and changes in the concentration of the constituents of the blood such as the protein, hemoglobin, and red blood cells Six of the patients had myasthenia gravis Of these, three were in a special research ward for periods of several months where esterase determinations were made practically every day, and often several times on the same day

METHODS

The method employed for the determination of the choline-esterase activity of the serum was McGeorge's modification (7) of the procedure of Stedman, Stedman and White (10) The method utilizes the amount of acid liberated in the hydrolysis of acetylcholine by the serum as an index of the activity of the choline-esterase. The serum was added to a substrate of acetylcholine bromide in a rubber stoppered flask which was kept suspended in a water bath at 30° C. The acid set free by the enzyme was neutralized by continuous titration with alkali which was added from a microburette by means of an intravenous needle which pierced the rubber stopper in the flask. The number of cubic centimeters of 1/100 N NaOH needed to keep the pH of the solution at 8.0 during the period of 20 minutes is the unit used in this report for expressing the choline-esterase activity All determinations were made in duplicate. In each instance simultaneous determinations of the spontaneous cleavage of duplicate samples of acetylcholine substrate were made. The results of these determinations are at variance with those of McGeorge who obtained such constant values that he later dispensed with the blank determinations and assumed a constant and small figure. In these studies the results obtained were invariably higher than those assumed by McGeorge. Furthermore, they varied not only with different samples of acetylcholine but often with specimens removed from the same bottle. Antopol, Tuchman and Schiffrin (11) likewise found that the spontaneous hydrolysis of the acetylcholine varied even when the samples were obtained *from the same bottle.*

OBSERVATIONS

The data on the choline-esterase activity of the sera of 109 subjects are given in Table I About 24 per cent of the subjects had serum esterase values of between 2.0 and 2.5, and an almost like number had values of between 2.5 and 3.0 In about 18 per cent of the [illegible]

TABLE I

The serum choline-esterase activity of 109 patients

Patient	Diagnosis	Sex	Age	Body weight	Choline esterase activity of serum	Remarks
			years	*kgm*	*cc 1/100 N NaOH*	
J S	Bronchial asthma	M	57		4 73	
A S	Hypertension Mild cardiac failure	F	61	70	4 34	
R A	Myotonia atrophica	M	45	65	4 25	
L S	Paralysis agitans	F	35	70	4 09	
	Myositis	M	27		3 75	
	Normal blood donor	M	36	80	3 56	
	Normal blood donor	M	37	80	3 49	
L D	Hyperthyroidism	F	51	62	3 47	
E L	Infectious arthritis	M	20	63	3 42	
A. S	Rheumatic heart disease Infarct of lung			62	3 40	
A. L	Hypertensive heart disease	F	70	52	3 26	
C P	Chronic hephritis	M	64	57	3 25	Ambulatory Blood urea nitrogen 55
N	Neurasthenia	M	31	75	3 20	
B S	Anxiety neurosis	F	63		3 16	
R. H	Epilepsy	F	13	30	3 13	
E S	Cirrhosis of liver	F	70		3 12	Serum albumin 2 0, serum globulin 5 1, R B C 2 8 million, Hb, 11 8 mgm per 100 cc.
E M	Progressive muscular dystrophy	M	9	37	3 00	Advanced muscular wasting Bedridden
S N	Bronchial asthma	F	15		3 00	
C C	Bronchial asthma	F	30		2 97	
D H	Epilepsy	M	35	78	2 97	
A. D	Peroneal muscular atrophy	F	43	73	2 95	
C L	Hyperthyroidism	M	51	55	2 95	B M R + 55 per cent
H A.	Arachnoiditis	M	31	51	2 94	
C	Hypertensive heart disease	M			2 83	
H M	Anxiety neurosis, obesity	F	30	80	2 78	
P S	Gonococcal arthritis	M	26	60	2 77	
L G	Progressive peroneal muscular atrophy	M	21	70	2 76	
G G	Progressive muscular dystrophy	M	25	70	2 75	
M J	Myasthenia gravis	F	32		2 70	Seriously ill
O O	Pulmonary tuberculosis	M	38	65	2 66	
E M	Carcinoma of breast with metastasis Cardiac decompensation	F	71		2 62	
M W	Carcinoma of rectum	F	68	46	2 61	
M K.	Progressive muscular dystrophy	M	23	66	2 60	Moderate disability
P L	Malignant hypertension	M	21	75	2 60	Died 2 days later
J C	Tabes dorsalis	M	58	67	2 58	
E R	Pemphigus vulgaris	F	67	53	2 57	Mild Afebrile course
R. W	Primary anemia	F	51	67	2 56	
R E	Acute tonsillitis	M	32	55	2 54	
J R	Hypertension, cardiac decompensation	M	54		2 53	
L R	Chronic arthritis	M	42		2 51	
C C	Peroneal muscular atrophy	M	29	75	2 50	
J D	Sciatic neuritis	M	32	71	2 49	
L L	Luetic heart disease	M	58	66	2 46	Edema, serum albumin 1 1, serum globulin 2 5 Three weeks later edema and serum albumin 0 9, serum globulin 3 2, serum esterase 2 51
H S	Hysteria	F	27	42	2 45	Poorly nourished
M N	Myasthenia gravis	F	37	60	2 43	Mild
A. S	Paralysis agitans	F	54	48	2 43	Violent tremors
E B	Multiple sclerosis	F	48	53	2 42	
S H	Myasthenia gravis	F	32	60	2 42	In remission
	Diabetes mellitus	F	66		2 38	
B L	Rheumatic heart disease	F	35	62	2 37	
S S	Sciatic neuritis	F	34	58	2 37	
M W	Arteriosclerotic heart disease	F			2 35	

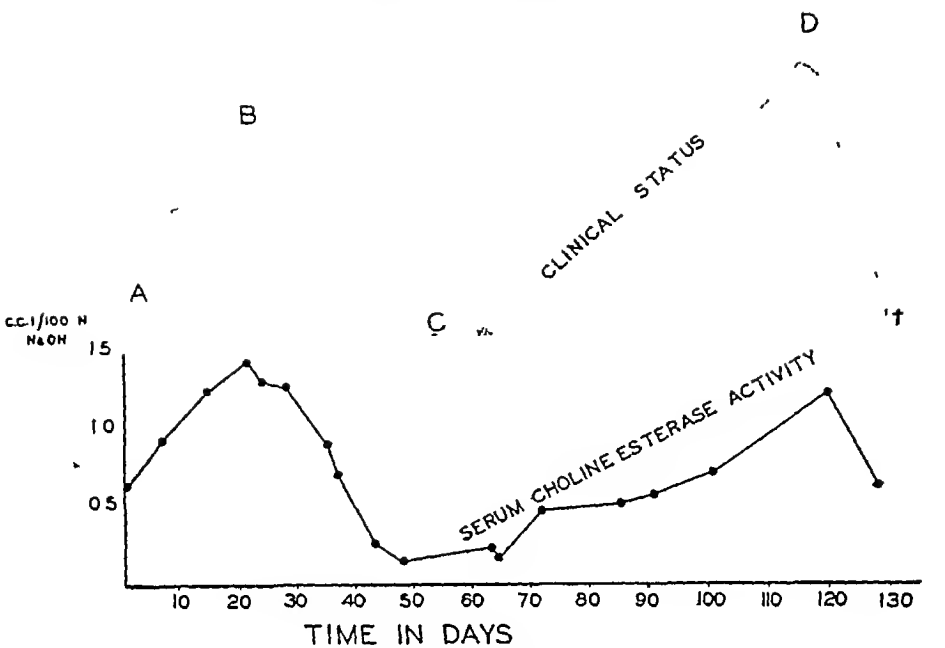


FIG. 3 CONCOMITANT CHANGES IN THE SERUM ESTERASE ACTIVITY AND THE CLINICAL STATUS OF PATIENT S S WITH PEMPHIGUS VULGARIS

Patient at A was bedridden at B he was able to walk about the ward, at C he was considerably debilitated and comatose, at D he was again much improved, at † the patient died.

with a value of 2.34 The animal showed no deleterious effects of the fast, its general condition was excellent although 27 per cent of the body weight was lost during the period of fasting

Convulsions The serum esterase activity in epilepsy was observed to be unchanged by a major seizure. The serum esterase activity of a girl aged 13 years with idiopathic epilepsy was deter- ... two hours before a convulsion, during a ...or seizure, and five minutes after the attack ... esterase values were 3 13, 3 13 and 3.24 ...

DISCUSSION

... observations made in these studies are in ... with those of Stedman, Stedman and ...on (6), Lucas, Hall, and Ettinger (5) and ... (7), in that no correlation was found the serum esterase activity and the clinical syndrome Nor did factors such as the age, sex, or body weight of the patient appear to have any effect on the esterase values In most subjects, including those with myasthenia gravis the esterase activity of the serum was constant over periods of weeks and even of several months However, in patients with debilitation, very low esterase values were observed In a few fatally ill patients with extreme debilitation the esterase activity of the serum was only about one-tenth to one-fifth that observed in normal subjects These values are comparable with those seen in normal persons after the administration of large doses of physostigmine or prostigmin in which instances the inhibition of the esterase activity is accompanied by considerable evidence of stimulation of cholinergic nerves Since most of the patients who showed such low esterase values in this study were entirely free from such symptoms

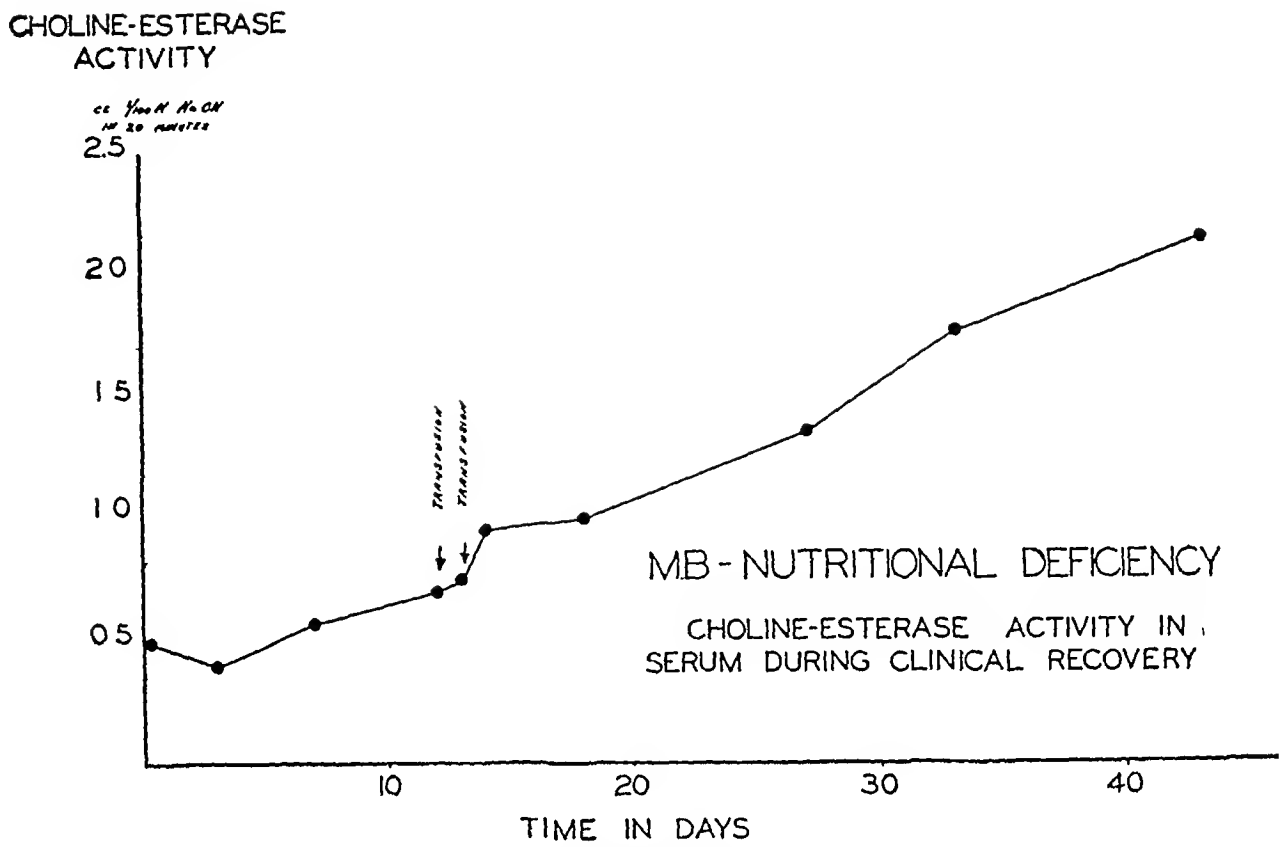


FIG 2 PATIENT M B WITH NUTRITIONAL DEFICIENCY

The esterase activity of the serum increased steadily as the patient's clinical condition improved

walk about the ward the esterase value was 1 42 During one period of exacerbation of symptoms when the patient was so seriously ill that his exitus was considered to be imminent the serum esterase activity was only 0 15 After considerable fluctuation in the clinical condition the patient finally died The esterase activity changed concomittantly with the fluctuations in the clinical condition of the patient During the last exacerbation of the symptoms the serum esterase value fell rapidly On the day before the patient died, the esterase activity of the serum was 0 63

Patient C S with chronic ulcerative colitis and severe debilitation had an esterase value of 0 66 Following a colostomy the patient showed a gradual and remarkable improvement in his general condition and the serum esterase activity increased to 2 05

Determinations of several constituents of the blood such as the red blood cells, hemoglobin, and serum proteins reveal no correlation between the concentration of these substances in the blood and the serum esterase activity Thus, in several patients wide changes in the concentration of these substances were not accompanied by any change in the serum esterase activity Furthermore, changes in the esterase activity in other subjects occurred without any consistent alteration in the amounts of these substances in the blood Numerous observations made on patients with fever showed no effect of changes in body temperature on the serum esterase value

Fasting The improvement in the clinical condition of Patient M B which was accompanied by corresponding changes in the serum esterase activity when large amounts of highly nutritious food were given suggested a study of the effects of fasting on the esterase activity The effect of deprivation of all food except water on the serum esterase activity was studied in a dog fasted for 24 days On the day preceding the fast the esterase activity was 2 13, on the eighth day of fast it was 2 19, and on the twenty-fourth day the esterase activity was practically unchanged

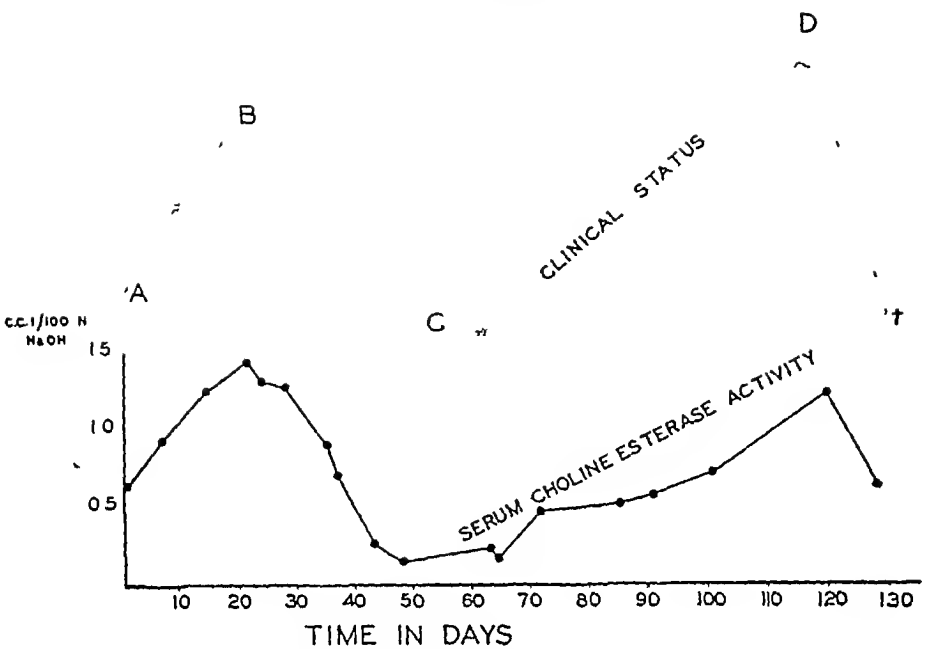


FIG. 3 CONCOMITANT CHANGES IN THE SERUM ESTERASE ACTIVITY AND THE CLINICAL STATUS OF PATIENT S S WITH PEMPHIGUS VULGARIS

Patient at A was bedridden, at B he was able to walk about the ward, at C he was considerably debilitated, and comatose, at D he was again much improved, at † the patient died.

with a value of 2 34 The animal showed no deleterious effects of the fast, its general condi tion was excellent although 27 per cent of the body weight was lost during the period of fasting

Convulsions The serum esterase activity in epilepsy was observed to be unchanged by a major seizure. The serum esterase activity of a girl aged 13 years with idiopathic epilepsy was determined two hours before a convulsion, during a major seizure, and five minutes after the attack The esterase values were 3 13, 3 13, and 3.24 respectively

DISCUSSION

The observations made in these studies are in agreement with those of Stedman Stedman and Easson (6), Lucas, Hall, and Ettinger (5) and McGeorge (7), in that no correlation was found between the serum esterase activity and the clinical syndrome Nor did factors such as the age, sex, or body weight of the patient appear to have any effect on the esterase values In most subjects including those with myasthenia gravis the esterase activity of the serum was constant over periods of weeks and even of several months However, in patients with debilitation, very low esterase values were observed In a few fatally ill patients with extreme debilitation the esterase activity of the serum was only about one-tenth to one-fifth that observed in normal subjects These values are comparable with those seen in normal persons after the administration of large doses of physostigmine or prostigmin in which instances the inhibition of the esterase activity is accompanied by considerable evidence of stimulation of cholinergic nerves Since most of the patients who showed such low esterase values in this study were entirely free from such symptoms

it is probable that acetylcholine was being produced in subnormal amounts at the nerve endings The lack of symptoms would indicate an equilibrium between the rates of production and cleavage of the acetylcholine This formulation assumes, of course, a relationship between the esterase activity in the serum and that at the nerve endings While no direct evidence of this relationship is available at the present time, it appears highly probable that such a relationship exists Whether the convulsions observed in one patient and the "myotonia" in another were related to a lowered rate of destruction of acetylcholine is not known The data show that convulsions do not affect the esterase activity of the serum Moreover, there is no evidence of an abnormal esterase value in idiopathic epilepsy In a series of observations to be published later the effect of physostigmine and prostigmin on convulsions will be discussed

Not all patients showing debilitation had low esterase values On the other hand, all of the patients with very low esterase values showed considerable debilitation subsequent to some advanced and in most instances generalized toxic process The patients in this group had a wide variety of clinical conditions, *e g*, nephritis, leukemia, carcinoma, nutritional deficiency, ulcerative colitis, pemphigus vulgaris, and lupus erythematosus In tuberculosis associated with cachexia Vahlquist (14) found very low esterase values That low esterase values are not characteristic of these conditions is shown by the higher values of other patients in the earlier stages of these diseases What factors in debilitation determine the lowered esterase activity (and presumably the lowered production of acetylcholine) are not known It is likely that any serious toxic state can decrease the production and hydrolysis of acetylcholine and that these changes are important factors in determining the degree of debilitation In contrast, patients seriously or even fatally ill with disease confined to one or a few organs but without generalized toxic manifestations showed only slight or moderate depression of the esterase activity Most patients studied in this investigation showed relatively little change in their serum esterase values over periods of several weeks On the other hand, some of the patients with debilitation showed considerable changes in the esterase activity of the serum concomitantly with changes in the clinical status In two such patients the serum esterase values were of prognostic significance

The serum esterase activity of the six patients with myasthenia gravis, in this series, was of the same order as that of most of the other patients without this condition The serum esterase values showed only minor, and apparently insignificant variations from day to day over periods of several weeks Furthermore, no correlation between the esterase value of the serum and the severity of the symptoms in myasthenia gravis could be established

It is of interest that the serum esterase activity showed no apparent relationship to the total mass of voluntary muscle Considerable reduction of the total muscular mass can take place without any change in the esterase activity of the serum, providing the patient is without a disease producing general debilitation

The data on the serum esterase activity of patients with hyperthyroidism, epilepsy, and anxiety states are not sufficient to permit a discussion of the results of Antopol, Tuchman, and Schifrin (11) and of Tod and Jones (15) Antopol, Tuchman, and Schifrin observed high esterase values in patients with untreated hyperthyroidism The few observations made in hyperthyroidism in these studies showed serum esterase values which were of about the same order as those in the other conditions investigated Tod and Jones found a high esterase activity in patients with anxiety states and low values in patients in catatonic stupor or with epilepsy The few determinations made in patients with epilepsy or with anxiety states in these studies showed no characteristic or unusual values in these conditions The present investigations suggest that the general physical condition of the patients must be considered in the evaluation of any group of data on the esterase activity in disease Furthermore, these studies indicate that the factor of debilitation is of more importance than is the nature of the clinical syndrome

SUMMARY

The choline-esterase activity of the blood serum in a large group of diseases was found to be unrelated to the type of clinical syndrome or to factors such as age, sex, body weight, muscular

mass, and body temperature. Convulsions and prolonged fasting were without effect on the serum esterase activity

In myasthenia gravis and muscular wasting the esterase activity of the serum was normal

The esterase activity differed widely among the subjects but was constant for periods of weeks in most subjects However, in patients with debilitation the esterase level often was low (one-fifth to one-tenth normal) and changed concomitantly with the clinical status of the patients

BIBLIOGRAPHY

1 Dale, H. H., Feldberg W and Vogt, M., Release of acetylcholine at voluntary motor nerve endings. J Physiol., 1936 86, 353

2. Brown G. L. Dale, H H., and Feldberg W., Reactions of the normal mammalian muscle to acetyl choline and eserine. J Physiol., 1936 87, 394

3 Walker, M. B., Treatment of myasthenia gravis with physostigmine. Lancet, 1934, 1, 1200

4 Walker, M B., Case showing the effect of prostigmin on myasthenia gravis Proc. Roy Soc. Med., 1934–35 28, 759

5 Lucas, C. C., Hall, G E., and Ettinger, G H., Individual and species variations in the choline esterase and other esterases of blood serum. J Pharmacol. and Exper Therap., 1935, 54, 151

6 Stedman E., Stedman, E., and Easson, L. H, Choline-esterase. An enzyme present in the blood-serum of the horse. Biochem. J., 1932 26, 2056

7 McGeorge, M., Choline esterase activity in disease—with special reference to myasthenia gravis Lancet, 1937, 1, 69

8. Hicks, C. S., Myasthenia gravis. Med. J Australia 1936 1, 894

9 Stedman, E. and Russell W R., The choline-esterase content of blood in myasthenia gravis Biochem. J., 1937, 31, 1987

10 Stedman E., Stedman E., and White, A. C., A comparison of the choline-esterase activities of the blood-sera from various species Biochem. J., 1933, 27, 1055

11 Antopol, W., Tuchman, L., and Schifrin, A., Cholinesterase activity of human sera, with special reference to hyperthyroidism. Proc. Soc. Exper Biol. and Med. 1937 36, 46

12. Shaffer, P, The excretion of kreatinin and kreatin in health and disease. Am J Physiol., 1908, 23 1

13 Milhorat, A. T., and Wolff H. G., Studies in diseases of muscle. I Metabolism of creatine and creatinine in progressive muscular dystrophy Arch. Neurol. and Psychiat., 1937 38, 992

14 Vahlquist, B., On the esterase activity of human blood plasma Skandinav Arch. f Physiol., 1935, 72, 133

15 Tod, H., and Jones M S., A study of the choline esterase activity in nervous and mental disorders. Quart. J Med., 1937, n.s 6, 1

THE EFFECT OF ARTIFICIAL PNEUMOTHORAX UPON THE ANOXEMIA OF PNEUMONIA[1]

By DAVID GOLDSTEIN, MORRIS BLOCK, AND MILTON ROSENBLÜTH

(From the Departments of Medicine and Physiology of the New York University College of Medicine and the Third (New York University) Medical Division of Bellevue Hospital New York City)

(Received for publication May 26 1938)

Statements concerning the usefulness of pneumothorax in alleviating the anoxemia of lobar pneumonia are few and conflicting, and are limited to estimates based upon the degree of cyanosis, the severity of dyspnea, and other criteria which are only roughly quantitative. It seemed that an examination of the effect of pneumothorax upon the degree of oxygen saturation of the arterial blood would afford more reliable information on the therapeutic value of this measure.

The following is a report on the oxygenation of blood in six cases of unilateral pneumococcus lobar pneumonia treated with pneumothorax. Diagnosis was based upon the usual clinical criteria and verified by x-ray Only cases in whom pneumothorax could be instituted within 72 hours of the onset of the disease were selected for study These were chosen irrespective of the type of pneumococcus Pneumococcus serum was given in only one instance. In establishing pneumothorax the technique of Blake (1) was employed, except that air was introduced at a rate of about 40 cc. per minute, and collapse of the lung was carried out as completely and rapidly as was consistent with the comfort and safety of the patient In certain cases mediastinal shift, adhesions, and massive consolidation limited the extent of collapse. Pneumothorax was performed in the lateral recumbent position, and inspiratory expiratory, and mean intrapleural pressures were recorded before and after the introduction of air

Arterial oxygen saturation was determined before the institution of pneumothorax, about two hours after separation of the pleura during several stages of collapse, and then throughout the disease and at less frequent intervals during convalescence. Serial roentgenograms were taken during the observation of each case by means of a 10 milliampere portable bedside unit Arterial blood was collected by puncture of the radial artery without novocaine.

METHOD

Ten cc. of blood were drawn into an oiled syringe containing sufficient powdered oxalate to make a 0.2 per cent solution. The blood was transferred without exposure to air and without negative pressure to a storage flask filled with mercury by means of a close fitting rubber junction Samples for analysis were transferred in a similar manner to a Van Slyke-Ostwald pipette. Equilibration with air was carried out in a mechanically rotated tonometer at room temperature. All analyses were made in duplicate by means of a Van Slyke manometric apparatus and according to the method of Van Slyke and Neill (2) The average deviation observed in duplicate oxygen determination was ± 0.25 per cent the precision of the saturation figures may therefore be taken as ± 0.5 per cent. We obtained 93.0 per cent (range 90 to 96 per cent) as the mean value for the arterial saturation in six normal subjects a figure in close agreement with the data recently published by Looney and Jellinek (3) Careful examination of the technique leads us to agree with these authors that the often quoted figure of 95 per cent for the saturation of normal arterial blood is erroneously high because of the practice of allowing the blood to stand in contact with oil. We find that the oxygen content of blood remains constant for a period of five hours when it is chilled immediately after collection and preserved over mercury whereas there is continuous diffusion of oxygen into the blood when it is preserved under oil at the same temperature, particularly when samples are being removed and the blood must be agitated. All blood gas analyses reported here were made within five hours of the time the blood was drawn.

RESULTS

A summary of the significant data in the six cases studied is presented in Table I Separation of the pleura was associated with a small rise in arterial saturation in two patients (T C and W T), with no change in two (W D and F J) and with a slight fall in [illegible] J [illegible] B T)

[1] This study was supported in part by a gift from Mr Bernard Baruch

per cent) The extent of final collapse estimated by x-ray varied from 30 to 90 per cent

2 Separation of the pleura (initial pneumothorax) was followed by (*a*) no change in oxygen saturation in two cases, (*b*) a fall in oxygen saturation in two cases, (*c*) a rise in oxygen saturation in two cases This group exhibited the most severe pleural pain, and the greatest relief after pneumothorax

3 Further collapse of the lung in no case was attended by an increase in oxygen saturation above the initial level In four cases, the oxygen saturation fell, after establishing collapse of the involved lung

PROTOCOLS

Case J J, Number 26243 (*Figure 1*) A 48-year old negro porter was admitted January 22, 1937, two days after onset with chilliness and generalized aches and pains There was no history of previous pulmonary disease. On physical examination, there was dullness over the right lower lobe, bronchial breathing, and showers of crepitant râles Sputum examination showed Type XIV pneumococcus In addition to the diagnosis of pneumococcus pneumonia, right lower lobe, the additional diagnoses of hypertensive and luetic heart disease with enlarged heart and dilated aorta were made.

Laboratory Blood cultures taken January 23 and 27 were sterile Leukocytes ranged between 20,000 and 30,000, granulocytes 90 to 94 per cent. Electrocardiogram showed left deviation of the electrical axis, and regular sinus rhythm

There was a fall in the arterial oxygen saturation in the presence of good collapse in the latter stages this may have been attributable to the development of a spread to the upper lobe. During the first days of fall, there was neither x-ray nor clinical evidence of new involvement. There was mild relief of pleural pain, with no associated rise in saturation on pleural separation. Pneumothorax did not prevent a fall in saturation in this patient

The patient died on February 1, 1937, the 13th day after onset.

At postmortem right lower lobe and more recent right upper lobe consolidations were found Additional findings were fibrinous pleuritis with effusion on the right, hypertensive and arteriosclerotic heart disease. The blood culture was negative.

Case B T, Number 26023 (*Figure 2*) A 35-year

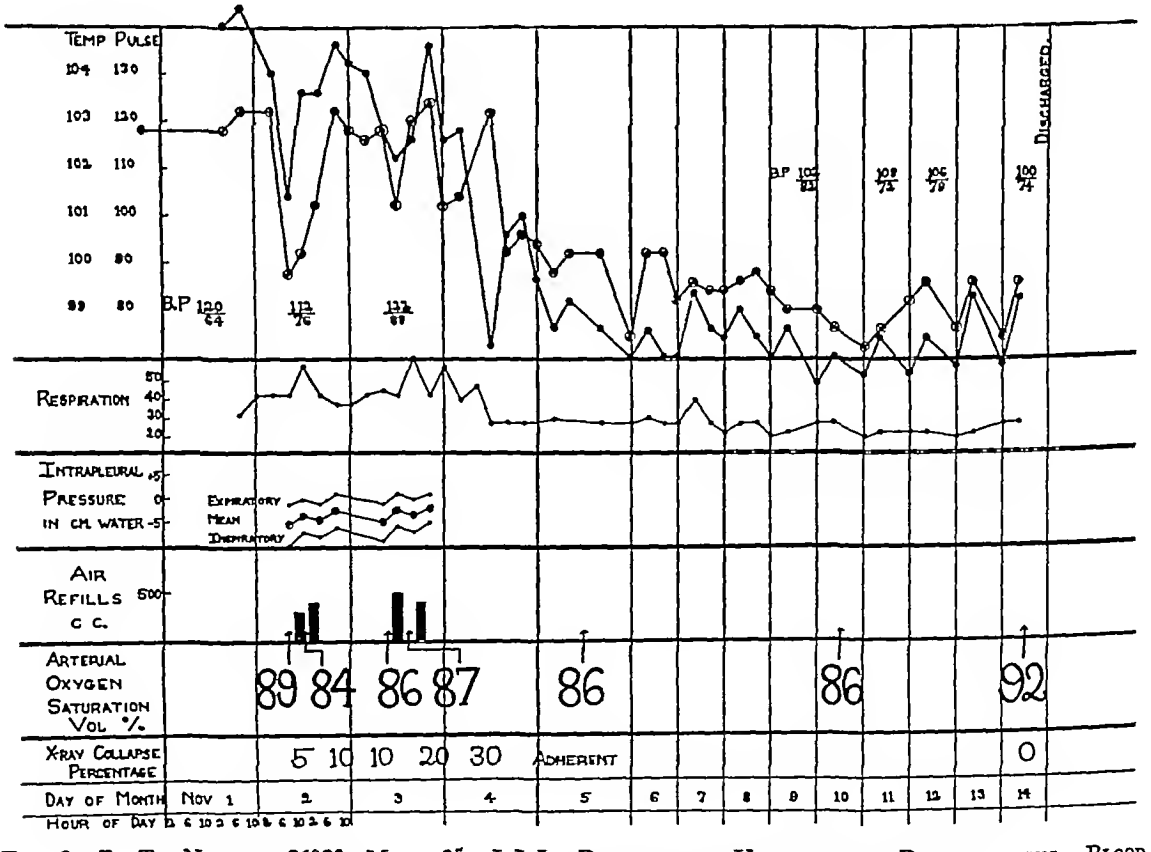


FIG 2 B T, NUMBER 26023, MALE 35 LLL PNEUMONIA. UNCLASSIFIED PNEUMOCOCCUS BLOOD CULTURE NEGATIVE. INITIAL PNEUMOTHORAX 54 HOURS AFTER ONSET

old handyman had the onset of his disease with chill, thoracic pain fever, cough, and scanty yellow expectoration at 7.30 a.m on October 31 1936 There was no history of previous pulmonary disease. Physical signs in the lung were dullness over the lower half of the posterior left chest, and showers of inspiratory râles.

Laboratory Blood culture taken November 1 1936 was sterile leukocytes 13,950 granulocytes 83 per cent. Sputum unclassified Pneumococcus Types I through XIV

Diagnosis Pneumococcus pneumonia of left lower lobe.

A very partial collapse was obtained. Crisis occurred on the fifth day Arterial oxygen saturation seemed relatively unaffected by pneumothorax. There was a persistence of unsaturation throughout the six days following crisis, despite good re-expansion. During this period chest signs were minimal.

Case F J Number 26281 (*Figure 3*) A white Polish housewife, 46 years of age, was admitted on January 6 1937 Two weeks preceding admission she had had a head cold and cough. On January 5 she expectorated a cupful of bloody sputum. She had generalized chest pain and several chills It was estimated that at the time of admission the pneumonia was of three days' duration. In the past history the patient was a known diabetic, taking insulin 15–0–0 without a well regulated diet. On physical examination there was dullness over the right lower lobe, without alteration in breath or voice sounds The abdomen was not distended.

Laboratory Sputum was thick and tenacious and Type I pneumococcus was found in the sputum. Leukocyte count was 15,850, with 74 per cent granular cells of which 23 per cent were stab forms hemoglobin 12.6 grams (87 per cent) R.B C. 3 47 million. The diagnosis of Type I pneumococcus pneumonia, right lower lobe, was made. Blood culture was negative throughout. The diabetes was controlled on a diet of carbohydrate 150, protein 65 fat 85 insulin 20–10–20 the latter being reduced to 5–0–0 before discharge. No serum was given.

There was progressive fall in arterial oxygen saturation within increasing collapse despite the absence of clinical evidence of mediastinal shift or pneumonia spread. On the third day of collapse acute respiratory distress supervened. At this time, the oxygen saturation was 69 per cent and the mean intrapleural pressure was + 2, respirations were 30 per minute, and cyanosis was intense. On removal of 600 cc. of air, mean intrapleural pressure was minus one cm. of water Twelve hours later, saturation in an oxygen tent was 80 per cent when the patient was removed from the tent, the saturation fell to 62 per cent. This episode was associated with a fall in temperature, which shortly after returned to normal.

Case T C Number 26124 (*Figure 4*) A 40-year

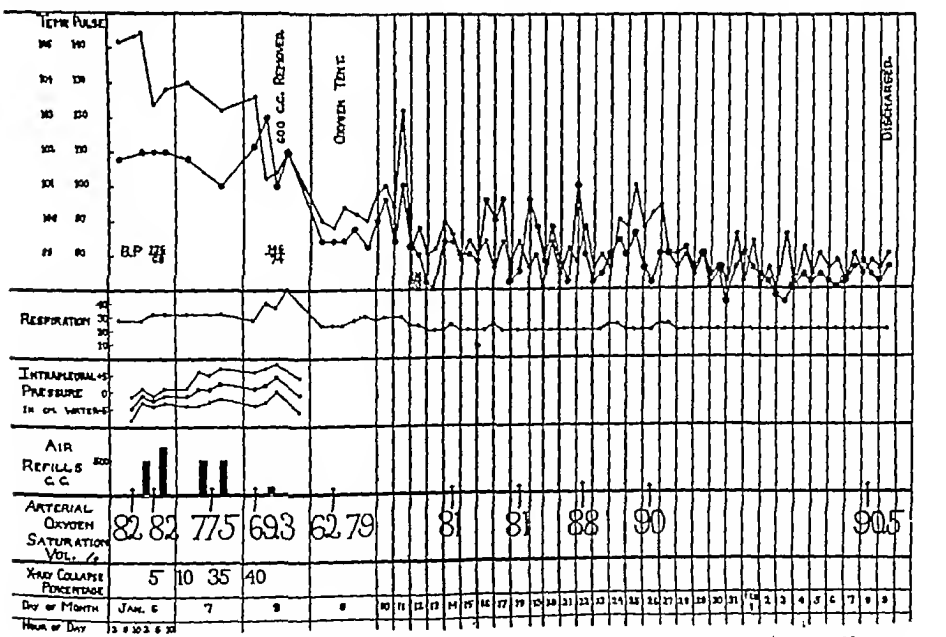


FIG. 3 F J., NUMBER 26281, FEMALE 46. R.L.L. PNEUMONIA. PNEUMOCOCCUS TYPE I BLOOD CULTURE NEGATIVE THROUGHOUT INITIAL PNEUMOTHORAX 72 HOURS AFTER ONSET

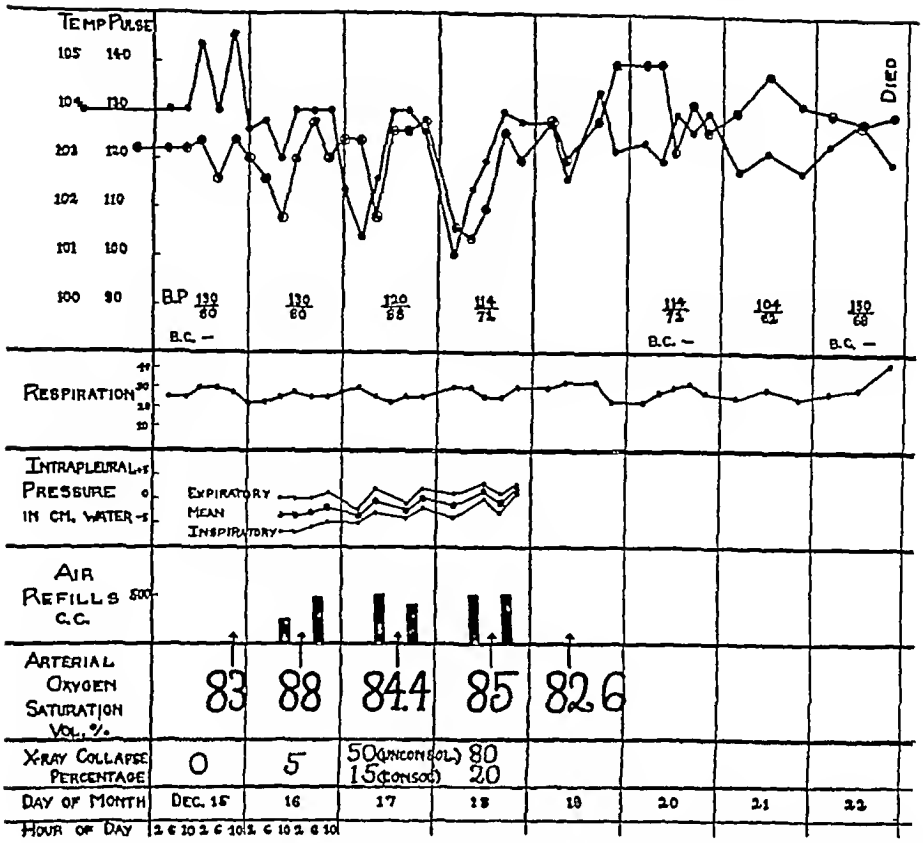
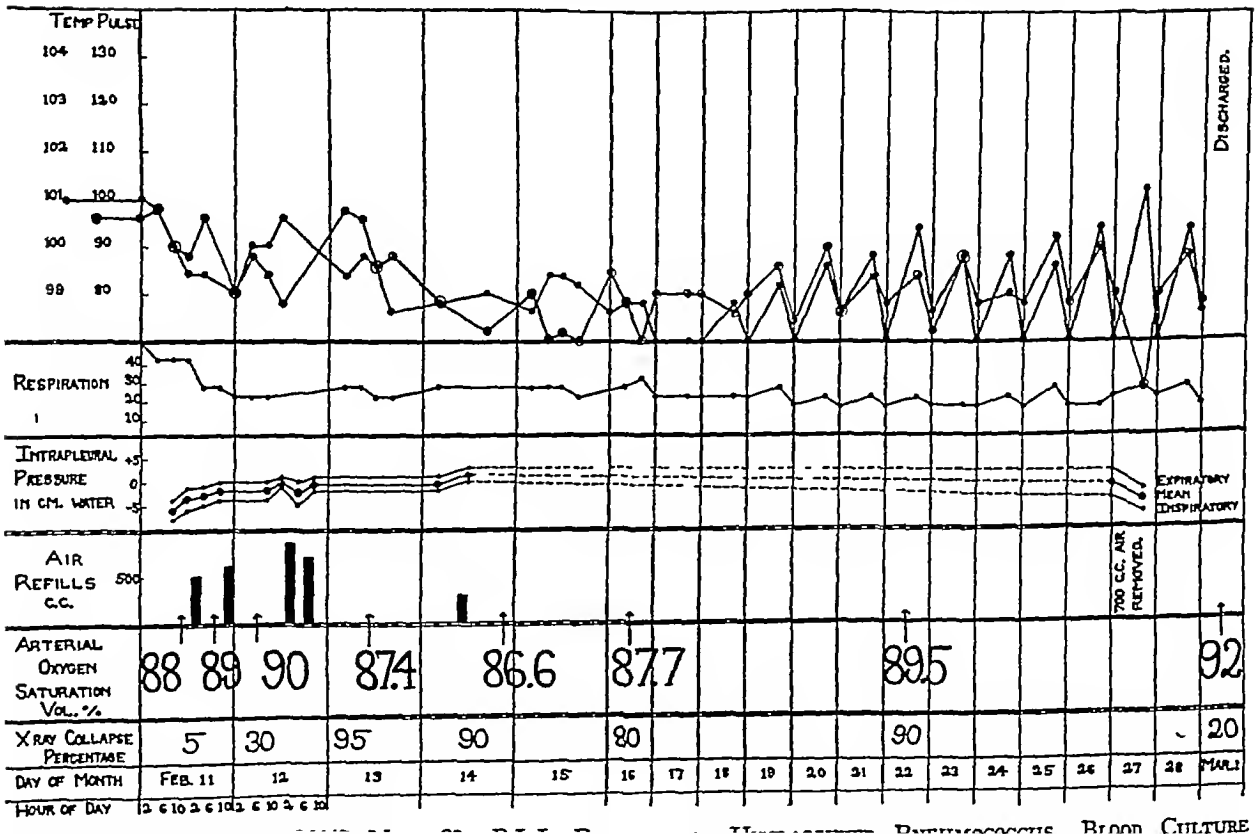


FIG 4 T C, NUMBER 26124, MALE 40 R L L PNEUMONIA. PNEUMOCOCCUS TYPE II BLOOD CULTURE NEGATIVE THROUGHOUT INITIAL PNEUMOTHORAX LESS THAN 72 HOURS AFTER ONSET TREATED WITH 200,000 UNITS TYPE II SERUM



old truck driver complained of malaise and mild pain over lower right axilla beginning December 13, 1936 and lasting to the time of admission December 15 1936. Chills, cough, and marked pleural pain appeared on December 14 1936 A convulsion on the morning of December 15, 1936 prompted hospitalization.

Past history was negative for previous pulmonary disease. Patient had experienced convulsive seizures for the past five years. Physical examination of the lungs revealed no abnormal findings. Fluoroscopy and x-ray revealed a definite shadow over the right lower lobe.

Laboratory Sputum tenacious and rusty, in which a Type II pneumococcus was found. Blood cultures December 15, 20, and 22 were sterile. Leukocytes ranged between 26 000 and 50 000, granulocytes 90 to 91 per cent.

Course 200 000 units Type II antipneumococcus serum were given

Diagnosis Pneumococcus pneumonia. Patient died on the 9th day of his illness

Associated with the relief of pleural pain following separation of the pleura, the oxygen saturation rose from 83 to 88 per cent. With subsequent refills however, the saturation fell There was 20 per cent collapse of the right lower lobe and 80 per cent collapse of the un involved lobes. Pneumothorax was discontinued because it was felt that consolidation interfered with further collapse. The final mean intrapleural pressure reading was + 2 cm of water The patient died. Permission for autopsy was not obtained.

Case W D Number 26442 (*Figure 5*) A 29 year old waiter awoke with a chill at 1 a.m. on February 11, 1937 followed by posterior right chest pain several hours later Slight cough was present, with no expectoration. Past history revealed nothing but "grippe" in 1935 Physical examination of the chest showed marked dull ness over right base posteriorly absent breath sounds, and no râles X ray showed a small shadow in the right lower lung field adjacent to the hilum.

Laboratory Unclassified pneumococcus, Types I to VIII and XIV were found in the sputum, leukocytes 13,000 to 27 000 82 to 88 per cent granular cells Blood culture taken February 11th was sterile.

Diagnosis Pneumococcus pneumonia of right lower lobe.

Despite complete collapse (90 per cent) unassociated with mediastinal shift, unsaturation continued for twelve days following fall in temperature.

Case W T, Number 26348 (*Figure 6*) A 46 year old steel worker complained of chill, chest pain cough fever and diarrhea of three days' duration. There was no expectoration. Headache and chest pain were very severe on admission. Past history was negative except for a chancre in 1905 inadequately treated.

Physical findings revealed dullness, diminished breath

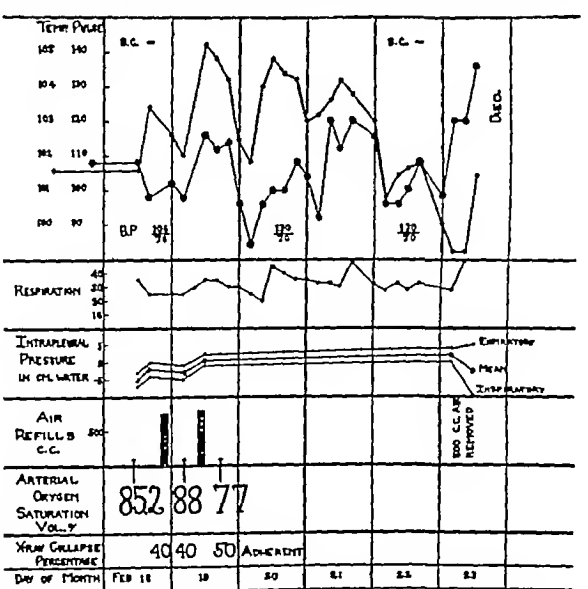


FIG. 6. W T., NUMBER 26348, MALE 46. R.U.L. PNEUMONIA. PNEUMOCOCCUS TYPE I BLOOD CULTURE NEGATIVE THROUGHOUT INITIAL PNEUMOTHORAX LESS THAN 72 HOURS AFTER ONSET

sounds, and a friction rub high in the right axilla Heart Position of maximal impulse was not felt. Sounds were of good quality A_2 was greater than P_2. There were no murmurs and the rhythm was regular X-ray showed a dilated aorta

Laboratory Blood cultures taken February 20 and 22 were sterile Leukocytes ranged from 19,950 to 39,000, granulocytes 93 to 95 per cent. Wassermann ±, sputum Type I pneumococcus

Diagnosis Pneumococcus pneumonia Type I of right upper lobe, luetic aortitis

Only 50 per cent collapse was obtained because of an apical adhesion (confirmed at postmortem examination) A transient slight rise in arterial oxygen saturation was obtained on separation of pleura, seemingly associated with definite relief of pleural pain Following the first refill on the second hospital day, saturation fell from 88 per cent to 77 per cent. There was no x-ray evidence of spread at this time, and no shift of the mediastinum. Death occurred on the sixth hospital day At postmortem examination there was an empyema on the right side, with spread of the pneumonia to the left upper lobe and left lower lobe. Syphilitic aortitis and aortic insufficiency also were found

BIBLIOGRAPHY

1 Blake, Francis G, Howard, Marion E., and Hull, Winifred S, Artificial pneumothorax in lobar pneumonia Medicine, 1936, 15, 1

2 Van Slyke, D D, and Neill, J M, The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I J Biol. Chem, 1924, 61, 523

3 Looney, J M, and Jellinek, E. M, The oxygen and carbon dioxide content of the arterial and venous blood of normal subjects Am J Physiol, 1937, 118, 225

4 Binger, C A L, and Davis, J S, Jr, The relation of anoxemia to the type of breathing in pneumonia. A study of respiration by means of a body plethysmograph. J Clin Invest., 1928, 6, 171

5 Binger, C A. L, and Brow, G R., Studies on the respiratory mechanism in lobar pneumonia, a study of lung volume in relation to the clinical course of the disease. J Exper Med, 1924, 39, 677

6 Binger, C A L, Brow, G R, and Branch, A., Experimental studies on rapid breathing II Tachypnea, dependent upon anoxemia, resulting from multiple emboli in the larger branches of the pulmonary artery J Clin Invest, 1924, 1, 155

7 Peters, J P, and Van Slyke, D D, Quantitative Clinical Chemistry Vol I Interpretations Williams and Wilkins Co, Baltimore, 1932, p 601

8 Binger, C A L., Anoxemia in pneumonia and its relief by oxygen inhalation. J Clin. Invest., 1928, 6, 203

9 Meakins, J C, and Davies, H W, Respiratory Function in Disease. Oliver, Edinburgh, 1925

BASAL GASTRIC SECRETION IN CASES OF PEPTIC ULCER
RELATION OF ACIDITY TO HEALING OF ULCER

By A. L. BLOOMFIELD and L. R. FRENCH

(*From the Department of Medicine, Stanford University School of Medicine San Francisco*)

(Received for publication June 1, 1938)

In studying gastric secretion it has been customary to promote the flow of juice by some sort of artificial stimulus, whether food or drug and such a procedure is of obvious importance where the digestive capabilities of the stomach are under consideration There is, however, another aspect of stomach function which is of value in clinical medicine, namely an assay of the secretions under resting or basal conditions Here one exposes the spontaneous play of autonomic nerves on the secreting cells as well as possible hormonic effects or "inherent" cellular activity, without the confusing element of external stimuli which promote maximal secretion and tend to wipe out individual differences It is obvious that the correlations of basal gastric secretion with disease may be quite different from those detected after the powerful stimulus of histamine.

METHODS

The technique of obtaining basal gastric secretion has been described elsewhere (1) Briefly, the patient is prepared as for a metabolism test, he is at rest in bed, warm, and he has taken no food for at least twelve hours A small tube is slipped into the stomach with the least possible disturbance and the fasting contents are withdrawn Continuous aspiration is then kept up over successive ten-minute periods until an approximately constant ten minute secretory volume is obtained—an indication that a basal level has been reached The entire test usually occupies from one to two hours In some subjects there is an obvious stimulation of secretion by passage of the tube, in others there seems to be a temporary inhibition Results in different patients are conveniently assessed by comparing the ten minute basal output of juice as well as its acidity, or the volume obtained over a longer period may be measured Table I gives the results in an illustrative case.

When measurements of basal secretion are carried out repeatedly on the same person a surprising constancy is observed although the findings vary greatly in different "normal" people (1) In one, both volume of secretion and acidity are low, in another, there may be a continuous abundant flow of highly acid juice, and in a third, there may be a small quantity of very acid secretion or *vice versa*. In those whose basal secretion is highly acid there is little further increase after a full dose of histamine—such a stomach is already working at nearly top speed (1)

TABLE I

Sample protocol of test for basal secretion in a normal young man

Number of specimen (10-minute period)	Character of gastric juice	Volume per 10-minute period	Free acid	Total acid
		cc.		
Fasting	Moderately bile stained mucoid material	30	5	20
1	Thin tinted fluid moderate amount of mucus	13	62	72
2	Same	14	73	82
3	Thin, clear colorless few particles of mucus	15	73	82
4	Same	15	73	82
5	Same	14	74	85

Basal secretion in peptic ulcer

The highly acid profuse secretion of patients with peptic ulcer has been repeatedly described, but the observations have usually been made after either a test meal or an injection of histamine (2) We have found no record of studies of basal secretion in peptic ulcer although it seems highly important to know about the "spontaneous" activities of the secreting cells in this disease.

Studies of basal secretion were made in twenty instances of peptic ulcer (11 duodenal, 9 gastric) The diagnosis was proved in case [illegible] ray operation or gastroscopy [illegible] of

this study (see below) we selected from a larger series cases in whom healing of the ulcer was very rapid (10 days to 3 weeks) or in whom the ulcer was quite refractory to therapy

RESULTS

In Table II are shown the acidity and volume of the basal secretion in these cases There are

TABLE II

Summary of findings in 20 cases of peptic ulcer

Gastric						Duodenal				
Name	Age	Sex	Size of ulcer	Basal secretion*		Name	Sex	Age	Basal secretion*	
				Volume	Acidity				Volume	Acidity
	years			cc.				*years*	cc.	
Tu.	45	F	"Small"	9	77	Wl.	M	46	16	140
Li.	51	M	1 cm.	20	66	Re.	M	35	30	136
Ma.	47	M	2.5×4.0 cm.	13	45	To	M	27	13	126
Lu.	61	M	2.5×2.0 cm.	4	44	Ca.	M	20	9	126
Le.	33	M	1.5 cm.	5	38	Ba.	M	38	11	120
Ko	35	F	2 cm.	14	0(free)	Ban.	M	39	13	98
Ch.	58	M	"Large"	19	0(free)	Sh.	M	33	13	80
Ku.	56	M	1 cm.	5	0(free)	Ho	M	52	18	75
						Ra.	M	46	8	72
						Gr	M	42	10	54
						Ha.	F	49	6	24

* "Volume" of secretion is the output obtained during a ten-minute period at the basal level. "Acidity" is total acidity as titrated in the usual way

several points of note First there is a marked difference in the rate of secretion and acidity of various cases, in contrast to the uniformly high values after histamine One-third of the cases of gastric ulcer had no free acid, one-half of the cases with duodenal ulcer poured out juice with an acidity as high as 120 to 140 even under basal conditions Decline of gastric acidity with advancing years has been pointed out by Polland (2), but his data were obtained by means of histamine tests or Ewald meals Relation of acidity to age is brought out very clearly in this series and is even more notable with juice secreted under basal conditions than with histamine juice The average acidity, for example, of five patients with duodenal ulcer with an average age of 47 years was 73, the acidity of five with an average age of 30 was 117 6 Four patients with gastric ulcer with an average age of 54 had acidity of 27 5, five with an average age of 38 had acidity of 52 5 In this small series there was no definite relation of volume of secretion to age among the cases with gastric ulcer, but such a relation is clearly seen in those with duodenal ulcer, the average volume in the five youngest patients being 15 2 cc. and in the five oldest 10 6 cc.

Of special interest seemed the great difference in acidity of the gastric and duodenal cases With histamine tests the average acidity of duodenal cases is slightly higher than that of the gastric, but there is no such discrepancy as appears when the basal juice is tested In this series, the average age of 9 patients with gastric ulcer was 47 years with average gastric acidity of 40 5, the average of 11 cases with duodenal ulcer was 39 with average acidity of 95 5, more than double that of the gastric cases The difference in age of 8 years of the two groups certainly could not account for the discrepancy, and these findings reinforce the feeling we have had for some time that gastric and duodenal ulcer are essentially different disorders

Relation of gastric acidity to healing of peptic ulcer

Reduction of gastric acidity is, in the minds of most physicians, the main objective in the therapy of peptic ulcer, and certainly it is hard to believe that healing of an eroded surface can proceed readily in a medium bathed in corrosive fluid But the situation is much more complex It is common knowledge, for example, that the bowel ulcers of typhoid disappear with amazing speed, when the infection has spent its force, even under a fecal current alive with bacteria So, too, everyone has seen deep peptic ulcers heal within two or three weeks despite an extremely high gastric acidity It may well be that the mucosa of the stomach is adapted to its acid bath in contrast to the jejunum which becomes eroded so readily if acid stomach contents are diverted into it by means of gastro-enterostomy Brown and Dolkart (3) have recently reviewed the subject and find no correlation between the course of peptic ulcer and gastric acidity, unfortunately, they used the Ewald test meal with which there is ordinarily so much variation that no conclusions can be drawn

It seemed of interest to investigate the subject from the standpoint of basal gastric secretion to

see whether any correlation exists between the course of peptic ulcer and the character of the spontaneous gastric secretion without complicating the situation by the use of any test meal or secretory stimulus

For purposes of analysis the groups of duodenal and peptic ulcer were further subdivided into those which healed promptly and those which were refractory to medical treatment consisting of rest —more or less complete—a simple dietary regimen, and belladonna. No systematic alkalinization was practiced Drugs were of course omitted before the basal juice was collected The results are best shown graphically (Figure 1) While

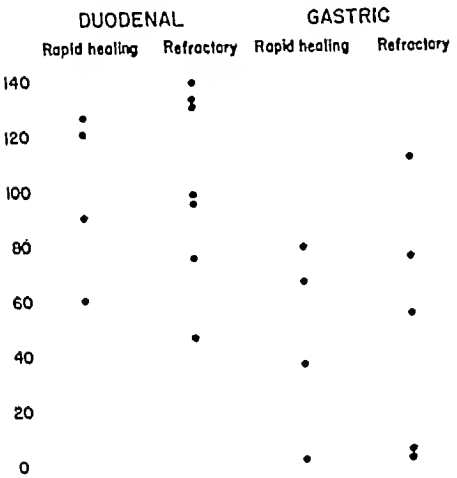


FIG. 1 RELATION OF HEALING OF PEPTIC ULCER TO BASAL ACIDITY

Each dot indicates the acidity in a single case.

the number of cases is too small for mathematical analysis, it is quite clear that in this series there is no relation between basal acidity and speed of healing In other words, ulcer may heal rapidly with high or low acid or may be refractory with high or low acid. As a matter of fact, the average acidity of the cases with duodenal ulcer with rapid healing was 95 the acidity of those which were refractory 96, with the ulcers of the stomach the corresponding figures were 37 and 48

In conclusion, we present two illustrative cases

Case 1 Rapid healing of ulcer despite high basal secretion M. B, a man aged 38 had had indigestion for about 8 years For the past three weeks the symptoms were constant and severe and he had passed black stools. Physical examination was not remarkable. Hemoglobin 78 per cent (12 grams per cent) X-rays (March 9 1937) showed marked six hour retention and great deformity of the duodenal bulb. There was rapid improvement of symptoms under usual therapy X rays (March 17 1937) showed no retention, bulb filled and was regular On June 4 1937, the patient was reported as being well. Test done on March 22, 1937 showed a basal acidity of 120

Case 2 Ulcer refractory to healing in spite of basal anacidity S G., a 58-year old man was seen in November 1936 for epigastric distress 6 years in duration. He had passed black stools and the hemoglobin was 48 per cent. X ray (November 6 1936) showed a large gastric ulcer on the lesser curvature. February 13 1937 in spite of treatment, x ray showed crater unchanged. Resection revealed a benign ulcer Test done February 16, 1937 at which time his hemoglobin was 75 per cent, yielded no free acid but large amounts of clear glairy mucus with the appearance and consistency of egg white. In spite of what one might expect to be a favorable medium for healing the ulcer was highly refractory to treatment.

COMMENTS

Attention is called again to the value of studies of basal gastric secretion as a supplement to the conventional test-meal methods The findings in cases of duodenal ulcer are of special interest since these patients for the most part pour out a continuous highly acid secretion, the average basal acidity in this group being 95 5, a value as high as that obtained in many normal controls even after histamine stimulation The average basal acidity of the cases with gastric ulcer, on the other hand, was much lower (40 5) Conventional views as to the relationship of acidity to the formation of ulcer and to healing are unfortunately not clarified by these observations which reveal no correlation between speed of healing and degree of acidity They seem to indicate that acidity is certainly not the major determining factor

SUMMARY

1 Studies of basal secretion in cases of peptic ulcer show that the average basal

denal ulcer was approximately twice as high as in gastric ulcer

2 Basal acidity in individual cases of peptic ulcer varied from 0 to 95, in duodenal ulcer from 24 to 140

3 There was no correlation between the degree of acidity and the speed of healing of either duodenal or gastric ulcer

BIBLIOGRAPHY

1 Polland, W S, and Bloomfield, A. L, Basal gastric secretion in man. Bull Johns Hopkins Hosp, 1931, 49, 302

2 Polland, W S, Histamine test meals An analysis of 988 consecutive cases Arch. Int. Med, 1933, 51, 903

3 Brown, C F G, and Dolkart, R E, Gastric acid during recurrences and remissions of duodenal ulcer Arch Int. Med, 1937, 60, 680

REQUIREMENTS FOR VITAMIN C IN MAN

By MARTIN HEINEMANN[1]

(From the Department of Internal Medicine Yale University School of Medicine New Haven)

(Received for publication June 2, 1938)

Tests for requirements for vitamin C give varying results according to the criteria which are established, for example, there are the widely different requirements (*i*) for complete saturation and (*ii*) for protection against scurvy In guinea pigs, doses of ascorbic acid which assure normal growth curves and freedom from scurvy are not sufficient to protect against the early pathological changes caused by deficiency of this vitamin (1, 2, 3, 4) It has been shown also that these animals are protected against scurvy by very small doses of vitamin C although their tissues contain scarcely detectable amounts of this vitamin (5, 6) In man, also, the daily dose of ascorbic acid adequate to prevent scurvy is far less than that required to maintain saturation of the body In this paper, the latter amounts, *viz*, the doses used by saturated subjects under normal and pathological conditions, have been investigated.

If a previously saturated subject is resaturated after having omitted all vitamin C for a certain length of time, the dose of ascorbic acid required to produce saturation corresponds to the quantity used from the body stores during the vitamin C-free period Consequently, this amount when divided by the number of days the experiment lasted, can be considered as the daily expenditure (7, 8) Saturation, as referred to in this paper, is defined as the condition when considerable portions of a moderate test dose of ascorbic acid appear in the urine. Under these circumstances the tissues contain maximal quantities of ascorbic acid and, in man, the concentration in whole blood is 14 to 15 mgm per liter Unless maximal amounts have been stored, the body retains administered ascorbic acid avidly, beyond this point of saturation, however, any excess of ascorbic acid is rapidly wasted into the urine a fact on which the saturation test is based (9, 10, 11) As stated before, the estimation of the daily requirements of vitamin C depends necessarily on the criteria applied

[1] Honorary Fellow

HEALTHY SUBJECTS

At one extreme, about 0 4 mgm per kgm daily is sufficient to protect against abnormal capillary permeability resulting from deficiency of vitamin C (12, 13, 14) At the other extreme more than 0 8 mgm per kgm is metabolized daily by a subject saturated with ascorbic acid (7, 8) All variations are possible between these extremes By his method, van Eekelen has demonstrated that the further removed from saturation the subject is, the smaller is the amount metabolized daily The amounts required to produce saturation have been calculated as 0 4 mgm per kgm after 94 days, as about 0.55 mgm per kgm after 40 days, and as about 0 8 mgm per kgm after 27 days of vitamin C deprivation (7, 8) Probably even more than 0 8 mgm per kgm would be metabolized at complete saturation levels That for the maintenance of a lower level of vitamin C reserves less is required is shown in another way by the following experiment A subject (78 kgm.) partly depleted as the result of a diet devoid of vitamin C, was supplied with about 0.5 mgm per kgm (40 mgm) daily *per os* for 16 days The concentration of ascorbic acid in whole blood had not changed during this period (5 4 mgm per liter on February 28, 5 5 mgm per liter on March 16) In whole blood (56), an ascorbic acid concentration of 14 to 15 mgm per liter indicates saturation, about or less than 2 mgm per liter have been observed in scurvy This small intake of about 0 5 mgm per kgm prevented further decline in the blood level That it fell far short of causing saturation is demonstrated by the failure of a test dose of 750 mgm of ascorbic acid, taken as a single dose *per os*, to cause any increase in the urinary excretion While from this observation 0 5 mgm per kgm can not be considered to cover maximal demands and to produce saturation in a depleted organism it appears that this dose just maintains a blood level of about 5 to 6 mgm per liter, at which level no clinical manifestations of scurvy have as yet been observed.

There is experimental evidence that 0 4 mgm per kgm daily represents the minimal requirement only and does not meet maximal demands A subject was saturated and thereafter supplied daily with 0 33 mgm per kgm for 32 days (15), and on another occasion with 0 5 mgm per kgm for the longer period of 53 days (16) In both instances a total of 16 mgm per kgm was necessary for resaturation The blood content had fallen from the saturation level of 14 to 15 mgm per liter to 7.2 mgm and 6 4 mgm per liter respectively Under both these experimental conditions, requirements calculated according to the method described in principle above also amount to 0 82 mgm per kgm (17)

These maximal requirements of 0 8 mgm per kgm daily may represent somewhat more than the optimum because there is no clinical evidence that a smaller supply, *e g*, 0 6 mgm per kgm has any deleterious effect Furthermore, it has already been shown that the amount of ascorbic acid used by the body decreases to some extent when the body stores diminish

Table I summarizes the results of determining maximal requirements in normal adults (8, 17, 18, 19), including one subject (J) with scurvy (19) Results agreeing closely to 0 83 to 0 84 mgm per kgm were obtained by calculating the weights for the experimental subjects from a weight-height-age table (20) As can be seen, maximal requirements in man are correlated with weight, this has been observed also for minimal requirements in man (14) and for guinea pigs (4) It has been suggested that requirements are comparatively larger in children than in adults (21, 22, 23), they are not larger in young guinea pigs than in old ones (4) O'Hara and Hauck (24) investigated the storage of vitamin C in 4 normal adults, their data (kindly supplemented by a personal communication) also indicates that the optimum requirement is considerably greater than the amount of vitamin C necessary to prevent scurvy Thirty mgm of ascorbic acid have been postulated as the daily minimum, 50 mgm as the daily optimum for the German population (25) There is some evidence that requirement for vitamin C varies with the total metabolism (26, 27, 28, 29), but it is apparently immaterial whether the calories are supplied by protein or carbohydrate (18)

TABLE I

Vitamin C requirements of normal subjects

Subject	Age	Weight	Daily requirements of ascorbic acid	
	years	*kilos*	*mgm*	*mgm per kgm*
v E	30	90	63	0 70
v W	38	72	56	0 77
J	18	53	44	0 83
El	25	68	53	0 78
			52	0 76
H	37	75	63	0 84
			63	0 84

From the foregoing, it follows that at least 0 8 mgm per kgm of ascorbic acid is used daily by a saturated subject Smaller doses, *e g*, 0 5 mgm per kgm or even less, are sufficient to protect against scurvy But, even if it is true that smaller doses may assure good health under optimal conditions, it would seem useful to supply the maximum requirements as a factor of safety against altered circumstances which may increase requirements

DISEASED SUBJECTS

An abnormally high supply seems to be needed in many diseases (30, 31, 32, 33, 34, 35), but this does not appear to be specific for any one disease So far, the knowledge concerning ascorbic acid requirement in disease is very incomplete, the methods applied to the problem have not always been adequate (36) Data obtained by the method of van Eekelen on a small group of patients are presented in Table II From the table it follows that requirements are unusually high in cases of tuberculosis (37, 38) This agrees with observations by Heise and Martin (39) who, by a different method, found that 55 to 138 mgm of ascorbic acid daily were required by a group of 44 tuberculous patients These authors and others (40, 41, 42) also observed that the stores of vitamin C in the tuberculous organism are very insufficient Fever has been considered as an important factor causing exhaustion of body stores (33, 35, 43), this may be referable to the increased total metabolism during fever On the other hand, it appears from observations of Patients N and v D that requirements can be increased in spite of normal body temperature The

TABLE II

Vitamin C requirements of diseased subjects

Subject	Diagnosis	Weight	Daily requirements of ascorbic acid	
		kilos	*mgm.*	*mgm. per kgm*
K.	Active pulmonary tuberculosis high temperature	59	82	1 4
L.	Active pulmonary tuberculosis, high temperature	55	139	2 5
V *	Active pulmonary tuberculosis high temperature	50	110	2.2
J *	Active pulmonary tuberculosis high temperature	44	93	2 1
N	Tuberculosis (spondylitis), normal temperature	72	86	1 2
E	Healed tuberculosis normal temperature (pleurisy 7 months ago)	55	45	0 8
v D *	Empyema following pneumonia, normal temperature	44	90	2 0
v E *	Empyema following pneumonia low grade fever	45	57	1.3
Th	Osteosclerotic (?) anemia Normal gastric acidity B M R. −5 low grade fever	76	121	1 6
St.*	Peptic ulcer	65	83	1.3
Bl *	Peptic ulcer	63	75	1 2
V O	Peptic ulcer	50	65	1.3
M	Peptic ulcer	69	83	1 2
R.	Peptic ulcer	?	85	?

* While as a rule, ascorbic acid was given by mouth, these patients received it subcutaneously in order to exclude the possibility that faulty absorption might only simulate increased requirements.

observation of normal requirements in a case (E) of healed tuberculous pleurisy without any symptoms of activity agrees with results of saturation tests from which Abbasy *et al* (34) concluded that body stores are normal in cases of quiescent surgical tuberculosis, while in active cases they are depleted. Increased requirements have been demonstrated also in tuberculous guinea pigs kept on a diet devoid of vitamin C, they develop scurvy earlier than a healthy control group (44, 45) A report from South Africa similarly shows that the incidence of scurvy among the natives rises with the morbidity from tuberculosis (46, 47) These observations indicate that *tuberculosis predisposes to scurvy by increasing the requirements for vitamin C* Amounts that will meet normal demands become inadequate.

It has been suggested before that the amounts required to maintain complete saturation may be even higher than those estimated by the method of van Eekelen employed for the present study This suggestion appeared to be substantiated by the following observation when a patient (J) was placed on his calculated daily amount for 2 weeks, the blood content decreased from 13 4 mgm. per 1000 cc. of whole blood to 8.7, at which level at least 600 mgm of ascorbic acid would have been needed for resaturation Abnormally increased requirements are not specific for tuberculosis as follows from determinations in other diseases (Patients v D, v.E, and Th)

Requirements have been found to be increased to some extent in patients with peptic ulcer, in whom similar experimental data were obtained by either oral or subcutaneous administration of the vitamin (Table II) Saturation tests have revealed that deficiency of vitamin C is rather common in this group of patients (32, 48, 49, 50, 51), but in only a few cases has manifest scurvy been described (35, 48, 49, 52)

Depletion was demonstrated also by measuring ascorbic acid in whole blood of hospitalized patients (Table III) who had been treated by the Sippy régime.

TABLE III

Milligrams of ascorbic acid per 1000 cc of whole blood

Subject	Peptic ulcer	Subject	Active tuberculosis
G	2.3	v W	2 7
B	1 8	J	5 7
H	1 8	v E.	6 7
St	1.8	N	9 4
Z.	1 8	L	3 0
R.	1 8	Br	3 1
L.	2.7	P	3.3
Go	1 8	v Z.	3 2
Bl	3 0	Vi	5 7
D	1 7	Je.	4.5
Mi	3 1	T *	1 8
Wi	2.5	v E.	3 8

* This patient had manifest symptoms of scurvy which were promptly influenced by ascorbic acid

Table III also presents for purposes of comparison, the ascorbic acid content in whole blood of a number of patients suffering from tuberculosis, whose requirements have been found to be greater than those of patients with peptic ulcer The values observed in the blood of tuberculous patients are low in spite of large amounts of orange juice taken for a few weeks previous to the determination of the vitamin in blood The low concentration of ascorbic acid in the

of patients with peptic ulcer, on the average even lower than in cases of tuberculosis, is owing chiefly to the dietary treatment, which obviously provides an insufficient supply of the anti-ascorbic vitamin In agreement with investigations in other countries (53, 54) pasteurized milk was found to contain between 0 4 and 1 0 mgm per 100 cc Since milk constitutes the main source of vitamin C in the diet commonly prescribed, the daily intake during the first and second week of Sippy treatment, for instance, amounts to no more than 12 to 15 mgm of ascorbic acid

As has been stated before, about 2 mgm or less of ascorbic acid per 1000 cc. of whole blood are observed in scurvy Yet, in spite of levels lower than 2 mgm per liter, none of these patients with peptic ulcers had any symptoms of scurvy, nor was capillary fragility increased The freedom from scurvy may be due to the fact that they were hospitalized and had almost complete rest. Exercise has been found to increase requirements and to exhaust the stores of vitamin C in rats (55) In this connection it is interesting to note that the only patient with scurvy—among the group with a blood level of less than 2 mgm—had entered the hospital, after several days of activity, considerably too fatiguing for his condition

Recently, requirements in two cases of peptic ulcer have been studied in a different way After the concentration of ascorbic acid in his whole blood (56) had been measured, one patient who could not take food by mouth received 100 mgm daily intravenously, another 60 mgm *per os* plus about 12 mgm daily with his diet After 6 days, repeated analyses of blood showed that in the first patient 100 mgm daily had not maintained the initial blood level, which had decreased from 9 3 mgm per liter to 7 0 mgm per liter The second patient had only a slight decrease (14 5 to 13 5 mgm per liter) His daily intake of about 75 mgm probably just covered his requirements Studied similarly, a patient with infectious mononucleosis with slight elevation of body temperature showed approximately normal requirements, the initial blood level of 14 0 mgm per liter rose to 15 3 after receiving daily for 6 days 1 2 mgm per kgm of body weight This method does not permit an exact quantitative evaluation of daily requirements Since it distinguishes between normal and distinctly increased requirements in a fairly simple way, it is proposed as a clinical method

The amount of ascorbic acid needed for saturation of the subject to be studied is estimated from the result of a preliminary analysis of whole blood (56) With a concentration of 8 mgm per liter of whole blood about 1000 mgm of ascorbic acid should be necessary, with 4 mgm per liter, about 2000 mgm (57) The amount presumably needed is supplied in single doses of 250 to 300 mgm Then, after an interval of about 12 hours, the concentration of ascorbic acid in whole blood is determined It should be about 10 to 12 mgm This initial saturation, necessary because maximum requirements are used only by a subject nearly saturated, is followed by a daily supply of the estimated normal requirements, 0 8 mgm per kgm or even more when larger requirements are assumed, for one week Another analysis of blood on the 8th day demonstrates whether the daily supply has been more than (rising concentration in blood), less than (decrease in blood), or just equal to the amount metabolized (no change in blood)

In this investigation, requirements in healthy subjects have been estimated from the effects of ascorbic acid administered *per os* It has been claimed that smaller doses, injected intravenously (58) or subcutaneously (59) are sufficient In a few diseased subjects, in whom the parenteral route has been chosen for certain reasons, the estimated requirements are about the same as when the vitamin is given by mouth.

SUMMARY

At least 0 8 mgm of ascorbic acid per kgm of body weight is used daily by healthy subjects saturated with this vitamin Smaller amounts maintain a lower concentration in the body stores To protect against scurvy, 0 4 mgm per kgm or even less per day appear to be sufficient

Abnormally high requirements are observed in patients with active tuberculosis, but are not specific for this disease

Requirements have been found to be increased to some extent in patients with peptic ulcer

A comparatively simple method for rough estimation of daily requirements is proposed

BIBLIOGRAPHY

1 Girond A., Ratsimamanga, R., Baratte, A. and Sylva, F., Réactions des animaux carençables à des doses croissantes d acide ascorbique. Compt. rend. Soc. de Biol., 1935 120, 701

2. Girond A. Leblond, C. P, and Ratsimamanga, R., The vitamin C requirement of the guinea pig Yale J Biol and Med. 1937, 9, 573

3 Zilva, S S., The ascorbic acid content of the intestine of the guinea pig Biochem. J., 1935, 29, 100

The effect of incomplete diets on the concentration of ascorbic acid in the organs of the rat. Ibid., 1936 30, 857

Vitamin C requirements of the guinea pig *Ibid.* 1936, 30, 1419

4 Dann, M., and Cowgill G R., The vitamin C requirement of the guinea pig J Nutrition, 1935 9, 507

5 Fox, F W., and Levy L. F., Experiments confirming the antiscorbutic activity of dehydroascorbic acid and a study of its storage and that of ascorbic acid by the guinea pig at different levels of intake. Biochem. J., 1936 30, 211

6. Bessey, O A., and King C G., The distribution of vitamin C in plant and animal tissues and its determination. J Biol. Chem., 1933, 103, 687

7 van Eekelen, M., Over opname, verbruik en uitscheiding van vitamine C door de mens. Thesis 1936, Drukkerij Fa Schotanus and Jens Utrecht.

8. van Eekelen, M., On the amount of ascorbic acid in blood and urine. The daily human requirements for ascorbic acid. Biochem J., 1936, 30 2291

9 Harris, L. L., Ray, S N, and Ward, A., The excretion of vitamin C in human urine and its dependence on the dietary intake. Biochem. J., 1933 27, 2011

10 van Eekelen M., Emmerie, A., Josephy, B., and Wulff L. K., Investigations about vitamin C. Acta brev Neerland., 1933 3, 168.

11 Johnson, S W., and Zilva, S S., The urinary excretion of ascorbic and dehydroascorbic acids in man Biochem. J., 1934 28 1393

12. Gothlin, G F., Methode zur Bestimmung der Festigkeit der Haut-capillaren und zu indirekter Beurteilung des individuellen *C vitamin standards.* Klin Wchnschr., 1932 11, 1469

13 Gothlin G F., Human daily requirements of dietary ascorbic acid. Nature, 1934 134, 569

14 Gothlin, G F, Frisell, E., and Rundqvist N., Experimental determinations of the *indispensable* requirements of vitamin C (ascorbic acid) of the physically healthy adult. Acta Med. Scandinav., 1937 92, 1

15 Hememann, M., Dietary influences on the amount of ascorbic acid and other *reducing substances* in urine. Acta brev Neerland., 1936 6, 67

16. Heinemann, M On the urinary excretion of thiosulphate and ascorbic acid during different diets. Acta brev Neerland., 1936 6, 141

17 Heinemann, M On the relation between diet and urinary output of thiosulphate (and ascorbic acid) II Human requirements for vitamin C. Biochem. J., 1936, 30, 2299

18. Heinemann, M., On human requirements for vitamin C under different conditions. Acta brev Neerland., 1936, 6, 144

19 van Wersch, H. J., Determinations of the daily requirements for ascorbic acid of man. Acta brev Neerland., 1936 6, 86

20 van Eekelen, M., Heinemann, M and van Wersch, H. J., On the daily requirements for ascorbic acid. Acta brev Neerland., 1936 6, 107

21. Szen Györgi, A., Die medizinische Bedeutung des Vitamins C. Deutsche med. Wchnschr 1934, 60, 556

22. Widenbauer, F., Ascorbinsaurestudien an Säuglingen. Klin. Wchnschr., 1936, 15, 815

23 Neuweiler W., Vitamin C Stoffwechsel bei Neugeborenen. Ztschr f Vitaminforch., 1937, 6, 75

24 O Hara, P H., and Hauck, H M., Storage of vitamin C by normal adults following a period of low intake. J Nutrition 1936 12, 413

25 Wachholder, K., Über die Vitamin C Bilanz des Menschen. IV Einschrankung des Bedarfes bei mangelhafter Zufuhr und deren ernahrungsphysiologische Bedeutung Klin. Wchnschr., 1938, 17, 5

26 Mosonyl, J., Skorbutische Stoffwechselstörung und Hyperthyreoidismus. Klin. Wchnschr, 1936, 15, 24

27 Svirbely, J L., The effect of desiccated thyroid, α-dinitrophenol and cortical hormone extract on the vitamin C content of some organs of the guinea pig fed graded doses of ascorbic acid. J Biol. Chem., 1935 111, 147

28. Kreitmair, H. Beiträge zur Pharmakologie der Ascorbinsaure (Vitamin C) Arch. f exper Path. u. Pharmakol., 1934, 176, 326.

29 Widenbauer, F., Der Vitamin C Haushalt des Menschen unter verschiedenen Verhältnissen. Klin. Wchnschr., 1937 16, 600

30. Mettier, S R., Mmot, G R., and Townsend, W C., Scurvy in adults Especially the effect of food rich in vitamin C on blood formation. J A. M A., 1930 95, 1089

31 Schroeder, H., Die Ausscheidung der Ascorbinsäure im gesunden und kranken Organismus. Klin. Wchnschr., 1935, 14, 484

32. Harris, L. J., Abbasy M A., Yudkin J and Kelly, S., Vitamins in human nutrition. Vitamin C reserves of subjects of the voluntary hospital class. Lancet 1936 1 1488.

33. Bullnwa J G M., Rothstein, I A., [illegible] D and Harde, E., Ceritamic acid [illegible] monias and some other pathni [illegible] Proc. Soc. Exper Biol. and Med.,

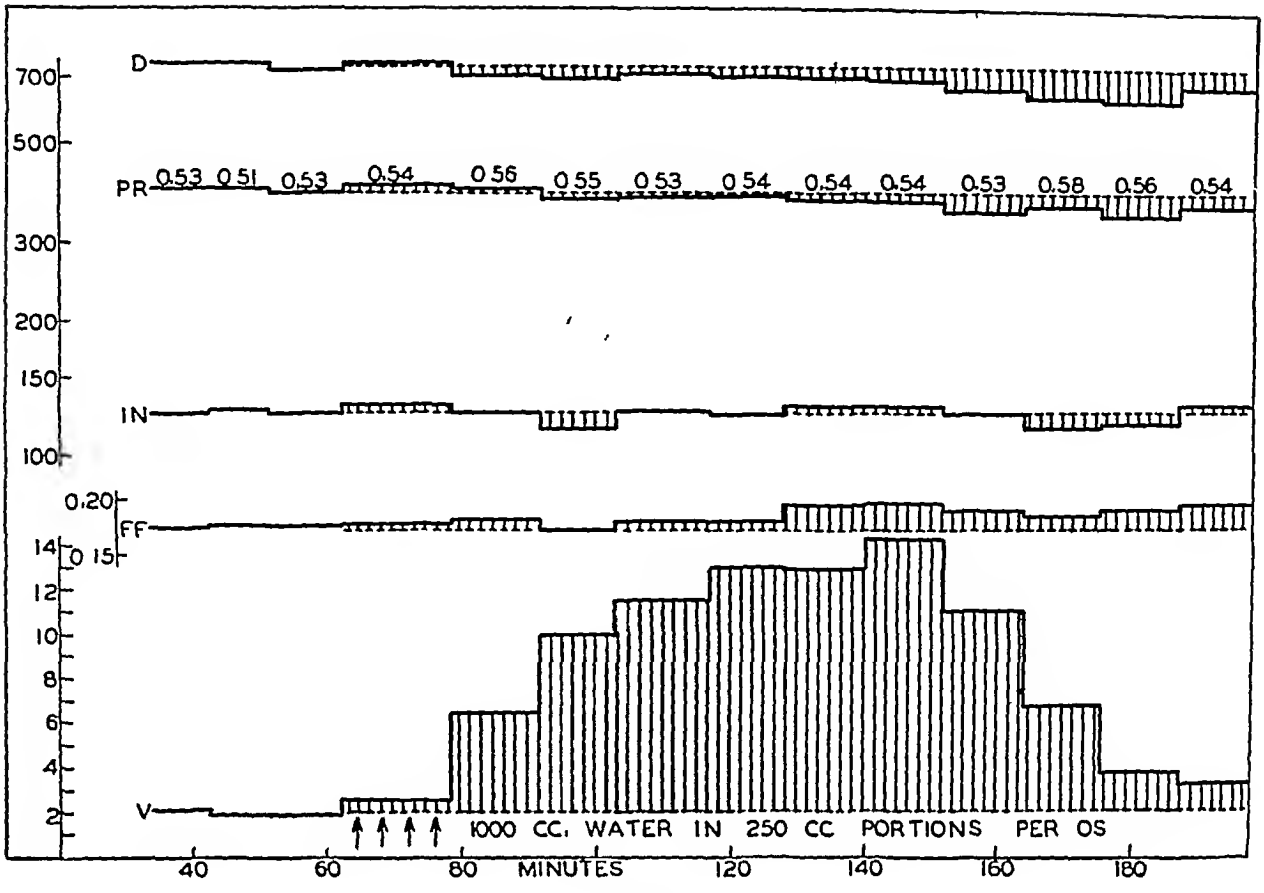


FIG 1

Data on Subject B L.

viewed elsewhere, that water diuresis is entirely referable to diminished tubular reabsorption and does not involve a change in renal blood flow or filtration rate (25)

Oil of juniper

(Figure 2, Subject R D, 1 8 sq m, 64 per cent plasma) This and other essential oils once enjoyed a vogue as diuretics (1) and our examination was made with this fact in mind In one instance, 0.2 cc. of oil of juniper was given orally, with no striking effect upon renal function except for a transient diuresis (2 3 to 6 3 cc per minute) In the observations recorded in Figure 2 one cc of oil of juniper increased the urine flow from 1 5 to 8 4 cc. (In this and subsequent figures the urine flow is recorded numerically at the bottom of the graph) It is doubtful if the increase in plasma flow is physiologically significant, and the filtration rate and filtration fraction remained practically unchanged In the absence of convincing evidence to the contrary, the diuresis could well be attributed to a decreased excretion of the antidiuretic hormone, perhaps in consequence of a centripetal stimulus from the gastro-intestinal tract

Figures 1 and 2 afford two series of control observations which, with numerous other instances that might be cited, warrant the conclusion that under basal conditions the plasma flow, filtration rate, and filtration fraction remain quite constant under continuous observation

Phlorizin

(Figure 3, Subject J C, 1 73 sq m, 58 per cent plasma) This glucoside produces inconstant but sometimes marked reductions in glomerular activity in all animals, and blocks the tubular reabsorption of glucose, xylose, and sucrose, and the tubular excretion of creatine and creatinine (25) Shannon (24) reported no reduction of the phenol red/inulin clearance ratio

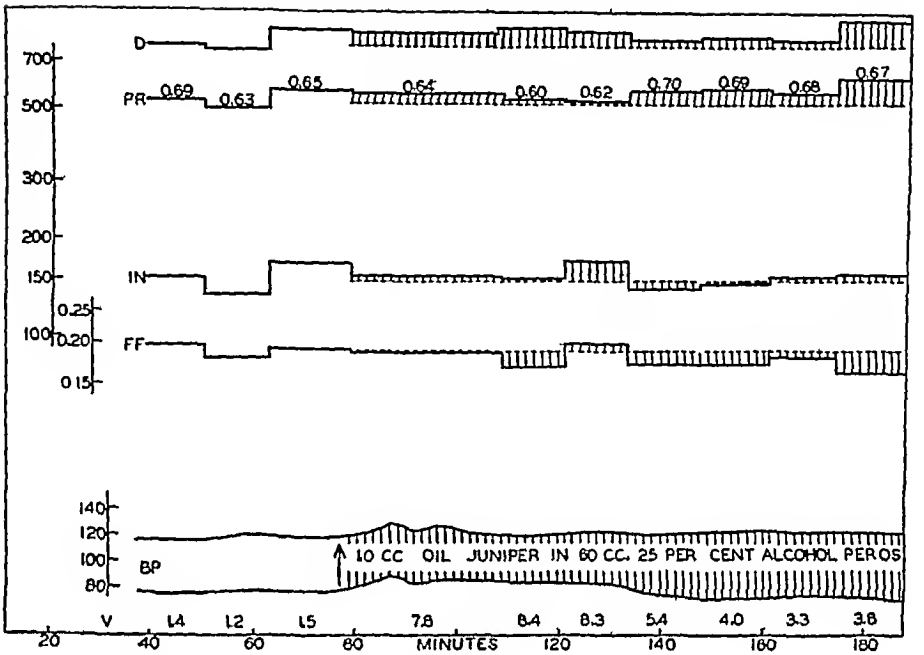


FIG. 2
Data on Subject R. D

in the phlorizinized dog, but Pitts' data (18) suggest that the drug may have some action on the tubular excretion of phenol red in the chicken

Our present observations demonstrate that, in man, phlorizin reduces the phenol red clearance to a much greater extent than the diodrast clearance, the excess reduction of the phenol red clearance being indicated in Figure 3 by the cross-hatched area. This fact indicates that the drug interferes with the tubular excretion of phenol red, and possibly also of diodrast. Since any clearance is acceptable as an index of renal plasma flow only so long as the renal A–V extraction ratio remains *constant, or very nearly so, it follows that* the administration of phlorizin invalidates the use of the phenol red clearance, and throws grave suspicion on the use of the diodrast clearance for this purpose The action of phlorizin raises the question of whether other physiologically reactive substances (adrenin, theophylline, *etc*) may not similarly modify the extraction ratio of diodrast, and thus invalidate the clearance method of following the renal blood flow This point is of such importance that it requires full discussion before applying this method further

The data of Table II of our previous paper (26) gave the phenol red/diodrast clearance ratio in 6 normal subjects as 0 56 Further data now available on 10 additional subjects leave this average at this figure, with the extreme variations of 0 46 to 0.73 In general, the ratio is very constant in any one subject, as shown in Figures 1 and 2, but there is a tendency for the ratio to fall when the diodrast clearance (plasma flow) is high, and to rise when the diodrast clearance is low This same inverse relation is evident in individuals in whom renal ischemia[1] or hyperemia

[1] The term "renal ischemia," is used here to denote any decrease in renal blood flow below normal in line with the use of the term, "renal hyperemia, to denote any increase above normal.

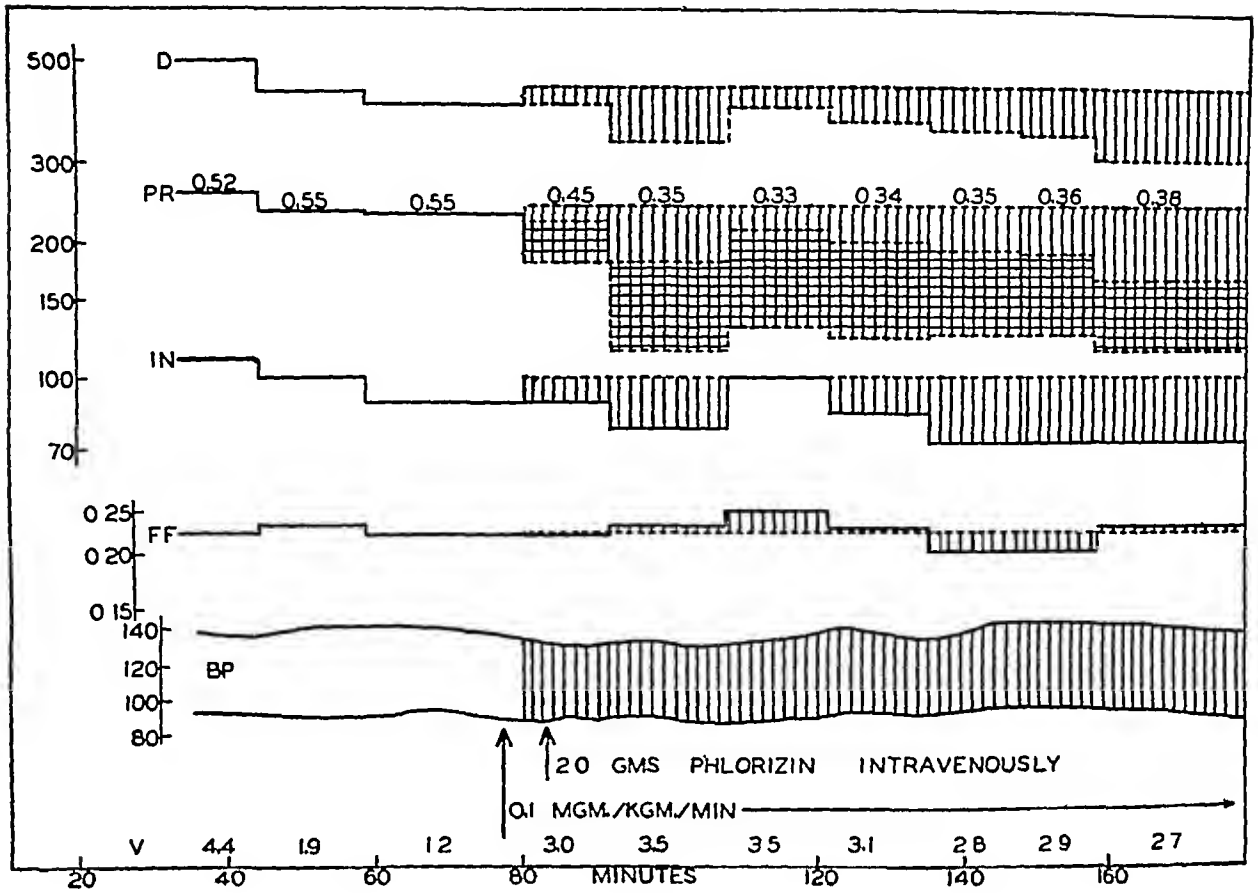


FIG 3

Data on Subject J C

has been induced artificially A further analysis of this phenomenon will be made subsequently, but an inverse relation between the phenol red/diodrast clearance ratio and the plasma flow is to be expected in theory for two reasons First, increasing the renal blood flow increases the quantity of both phenol red and diodrast presented to the tubules per unit time, this circumstance would have little or no effect upon the diodrast clearance, since the extraction ratio of diodrast is independent at these low plasma concentrations of the rate of delivery to the tubules, but increased rate of delivery of either substance would cause some reduction in the extraction ratio of phenol red, since this is limited in part by concentration factors within the excretory mechanism itself, and consequently the phenol red clearance would not increase proportionally to the blood flow Second, insofar as the phenol red extraction ratio is reduced below 100 per cent by the failure of phenol red to diffuse out of the peritubular capillaries in consequence of a reduction in diffusion gradient by protein-binding, or insofar as the ratio is reduced by temporal delay in the process of tubular excretion, prolongation of the renal circulation time would cause the extraction ratio to rise The diodrast extraction ratio, being close to 100 per cent, would be affected to a lesser extent, and so long as the vascular bed remained constant the phenol red clearance would approach the diodrast clearance during renal ischemia and fall away from it during hyperemia It may fairly be expected that, after the administration of a drug which brings about a change in renal plasma flow, the phenol red/diodrast clearance ratio should not deviate from its control value to a greater extent than is observed during equivalent changes in diodrast clearance in subjects who have received no medication

On the other hand, if the tubular excretion of phenol red and diodrast has been impaired, or interfered with by a competitive solute, there are

reasons to expect the phenol red clearance to be depressed to a greater extent than the diodrast clearance, in which case the clearance ratio would of course fall We have previously shown that diodrast and hippuran, which have very high tubular clearances, have a marked depressive action on the phenol red clearance, while phenol red has but a slight depressive action on its own clearance or on the diodrast clearance (26) And it has been shown elsewhere that iopax and neoiopax likewise specifically depress the phenol red clearance more than they depress their own clearance (27) These relationships, which are quantitative, reversible, and reproducible, indicate that phenol red is a much more sensitive indicator of the presence of a solute which competes for the tubular mechanism than is diodrast. We now add to this list, phlorizin, which probably depresses tubular activity for reasons other than those that specifically limit the excretion of phenol red, diodrast, *etc*, when in competition for the normal mechanism of transfer Here again the phenol red clearance is more sensitive to adverse action than is the diodrast clearance It is, of course, conceivable that some drug may be found which depresses the diodrast and phenol red clearances in precisely the same degree, but in the absence of knowledge of such a substance, and in the light of the above observations, we believe that the phenol red/diodrast clearance ratio is a sensitive index of interference on the part of any agent (drugs, hormones, *etc*) with tubular excretion, it being expected that such interference will be revealed by a fall in ratio when the diodrast clearance is decreased, contrary to the expectation that during a decrease in this clearance in consequence of true renal ischemia the ratio should rise With the knowledge gained from the action of phlorizin and the other evidence cited, we are in a position to interpret the action of other substances with greater certainty

The abnormal depression of the phenol red/diodrast clearance ratio in Figure 3, invalidating as it does the use of both clearances as indices of plasma flow, leaves us in the position of being unable to say with any certainty what effect *phlorizin* has upon the renal circulation The rise in the "apparent," filtration fraction is in keeping with the idea that the tubular excretion of diodrast has been slightly impaired. The action of phlorizin has been examined in one other individual, with results qualitatively the same as those shown in Figure 3

Adrenin

(Figure 4, Subject P V, 1 7 sq m, 57 per cent plasma.) In 1922 Richards and Plant showed that when the perfused rabbit kidney is supplied with blood at a constant rate of flow adrenin causes a rise in the perfusion pressure and at the same time swelling of the kidney, an observation confirmed in eviscerated rabbits and dogs (21, 22) Richards and Plant interpreted the paradox of simultaneous vasoconstriction with renal expansion by suggesting that adrenin acts preferentially to constrict the efferent arterioles, thus causing distension of the glomerular and preglomerular vessels This interpretation has been affirmed by Winton (35, 36) from observations on the heart-lung-kidney Thermostromuhr measurements on anesthetized, decerebrate dogs show that adrenin consistently reduces the renal blood flow (10 28, 31), though the threshold of the renal vessels in such animals is about 100 times as high as the threshold of the vessels of the muscles and skin (10)

We believe that observations on such preparations as are discussed above should be transferred to the normal organism with caution However, in this specific instance, our observations on normal man are in agreement with the conclusions reached from the above evidence As seen in Figure 4, 1 mgm of adrenalin given subcutaneously, with massage for two or three minutes immediately afterwards, caused a reduction in plasma flow from 680 cc. (1192 cc. of whole blood) to a minimum of 373 cc (654 cc. of whole blood) per minute The filtration fraction increased from 16 per cent to a maximum of 29 per cent If the reduction in plasma flow were owing to constriction of the afferent arterioles or of vessels proximal to these, it is to be expected that this constriction would reduce the effective glomerular pressure and therefore the filtration fraction, and a reduced filtration fraction and reduced plasma flow would lead to a reduction in the filtration rate. But if constriction occurs at the eff
(with or without dilatation of the

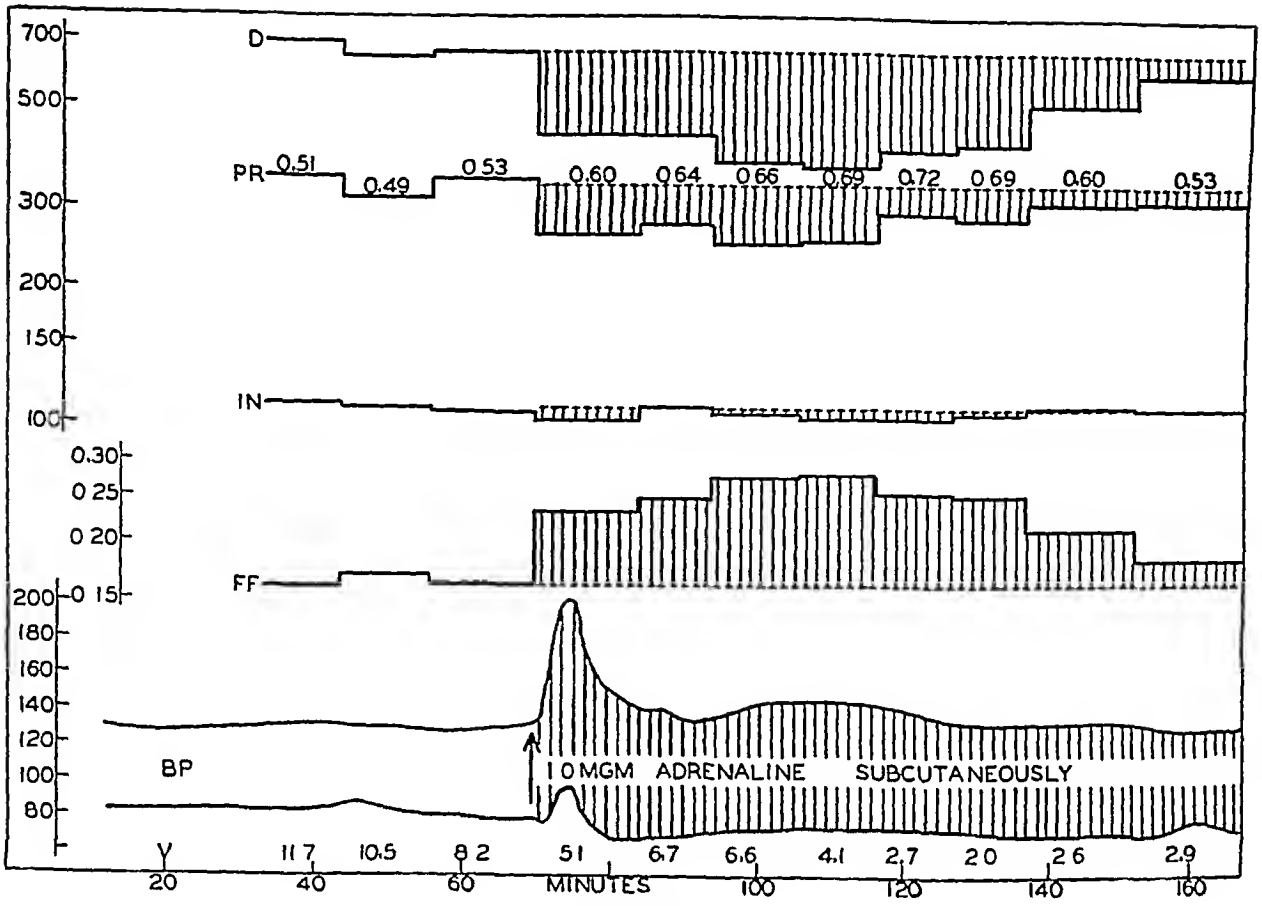


FIG 4
Data on Subject P V

oles),[2] it is to be expected that the effective glomerular pressure, and therefore the filtration fraction, would be raised more or less *pari passu* with the reduction in plasma flow, the increased filtration fraction would tend to offset the decreased plasma flow and to maintain the filtration rate at a constant level The action of adrenin is wholly consonant with the latter view

There appears to be only one alternative to the above interpretation if glomerular hemodynamics and the permeability of the glomerular membranes are such that a rigidly constant volume of fluid is filtered per unit time, regardless of glomerular pressure, then of course the filtration rate will re-

[2] Though the point is not established, one presumes that equilibrium between filtration pressure and the opposing osmotic pressure of the plasma proteins and the capsular pressure is reached by the time the plasma emerges from the glomeruli If equilibrium is not reached, then, of course, the time during which the plasma remains in the glomeruli, and therefore the volume of the glomerular capillaries and the rate of blood flow, must be considered along with the filtration pressure as determinants of the filtration rate. This consideration would not, however, alter the above statement.

It appears unnecessary to postulate that adrenin dilates the afferent arterioles, as does Winton (36) If the osmotic pressure of the plasma proteins is taken to be 25 mm. Hg and this is raised by filtration to 30 mm, and if the capsular pressure is taken to be 15 mm., the total pressure opposing filtration will be 45 mm. Taking the mean normal glomerular pressure as 60 per cent of the mean arterial pressure, the effective filtration pressure in the above subject would be 66 — 45 or 21 mm. Under the action of adrenin the emergent blood would have an oncotic pressure not exceeding 38 mm , at a constant filtration rate, and therefore a constant capsular pressure, the total pressure opposing filtration would be 38 + 15 or 53 mm. Taking the mean glomerular pressure as 90 per cent of the mean arterial pressure, the effective filtration pressure would be increased to 99 — 53 or 46 mm , enough to double the filtration fraction. An increase of 100 per cent is the largest we have observed in the normal subject before and after the administration of the adrenin.

main constant and independent of plasma flow, and the filtration fraction will vary inversely as plasma flow This alternative explanation, however, is very suspect First, it is not in harmony with the evidence that the separation of capsular fluid in the glomeruli is effected by an unconditioned process of filtration, rather than by a conditioned process of transudation, and this is too substantial to be lightly rejected (25) Any membrane which conditions the rate of passage of water regardless of hydrostatic pressure must, we believe, possess differential permeability to the electrolytes and other constituents of the plasma. Second, the alternative explanation is contrary to the fact that the filtration rate does, under certain circumstances, increase or decrease through a range of — 50 to + 100 per cent (see also Figures 3, 5, and 6)

We therefore reject the alternative explanation, and present the data in Figure 4 as substantiation of Richards and Plant's thesis for the normal human kidney (21, 22) These data further demonstrate that, in the normal kidney, the degree of efferent arteriolar tone, and therefore the effective filtration pressure, are submaximal and can be caused to approach maximal values by adrenin Presumably sympathetic activity has this same action on the efferent arterioles [8]

During the period of renal ischemia the phenol red/diodrast clearance ratio rises above its control values, as is to be expected in theory, the fact that this ratio is not reduced during the period of ischemia is evidence that adrenin has not specifically impaired the excretory activity of the tubules, and that the diodrast clearance is a valid indication of changes in blood flow

It has been our experience and the experience of others that adrenin may cause a marked oliguria. It is quite possible that this *oliguria is* central in origin, *i e*, release of antidiuretic hormone from the pituitary gland, since Rydin and Verney (23) have shown that the inhibition of water diuresis which is associated with emotional stress and exercise is explicable on this basis In anticipation of oliguria, the observations in Figure 4 were made on the descending limb of water diuresis, and 2 0 per cent Na_2SO_4 was incorporated in the infusion fluid in order to maintain the urine flow For this reason the data on urine flow have no special significance.

The action of adrenin has been examined in five other subjects with results qualitatively similar to those given in Figure 4 One of the most notable features in the action of this hormone is that the filtration rate remains remarkably constant in spite of relatively great changes in renal plasma flow This has been noted in every subject examined, and it suggests an important reciprocal relation between the diameter of the lumen of the efferent arterioles the blood flow, and the effective glomerular pressure. If the renal blood flow is controlled primarily by variations in the efferent arteriolar tone, wide variations in this tone would leave the filtration rate relatively unchanged.

Theophylline and caffeine

(Figure 5, Subject B L, 1 83 sq m, 55 per cent plasma) Caffeine causes an increase in the number of active glomeruli and engorgement of the glomerular vessels of the amphibian kidney when the latter is exposed for microscopic examination (25), but such observations do not have a conclusive bearing on the action of the drug in the mammalian kidney, with the exception of the rabbit, normal glomerular activity appears to be more constant and more nearly maximal in the mammals than in the lower vertebrates (6, 25)

The widespread opinion that caffeine and other purine derivatives typically induce hyperemia in the mammalian kidney rests upon four methods of observation measurements of arterial or venous flow by a mechanical stromuhr, measurements of the size of a kidney by the oncometer, measurements of the rate of flow in the perfused heart-lung kidney and measurements of blood velocity by a thermostromuhr attached to the renal artery or vein in an anesthetized or conscious animal (For the early literature on this subject see 2 and 4) Methods involving the use of perfused kidneys or eviscerated, anesthetized animals can be

[8] The rise in blood pressure immediately after the injection of adrenin is in this instance, exaggerated by the massage used to facilitate absorption. In other cases, this pressure rise has been less marked. It is typical of the action of moderate doses of adrenin in man that, while the systolic pressure is slightly increased the diastolic pressure is reduced. It would seem that this is caused by the reduction of the mean peripheral resistance by dilatation of the skeletal muscle and coronary arteries, simultaneously with an increase in the cardiac output.

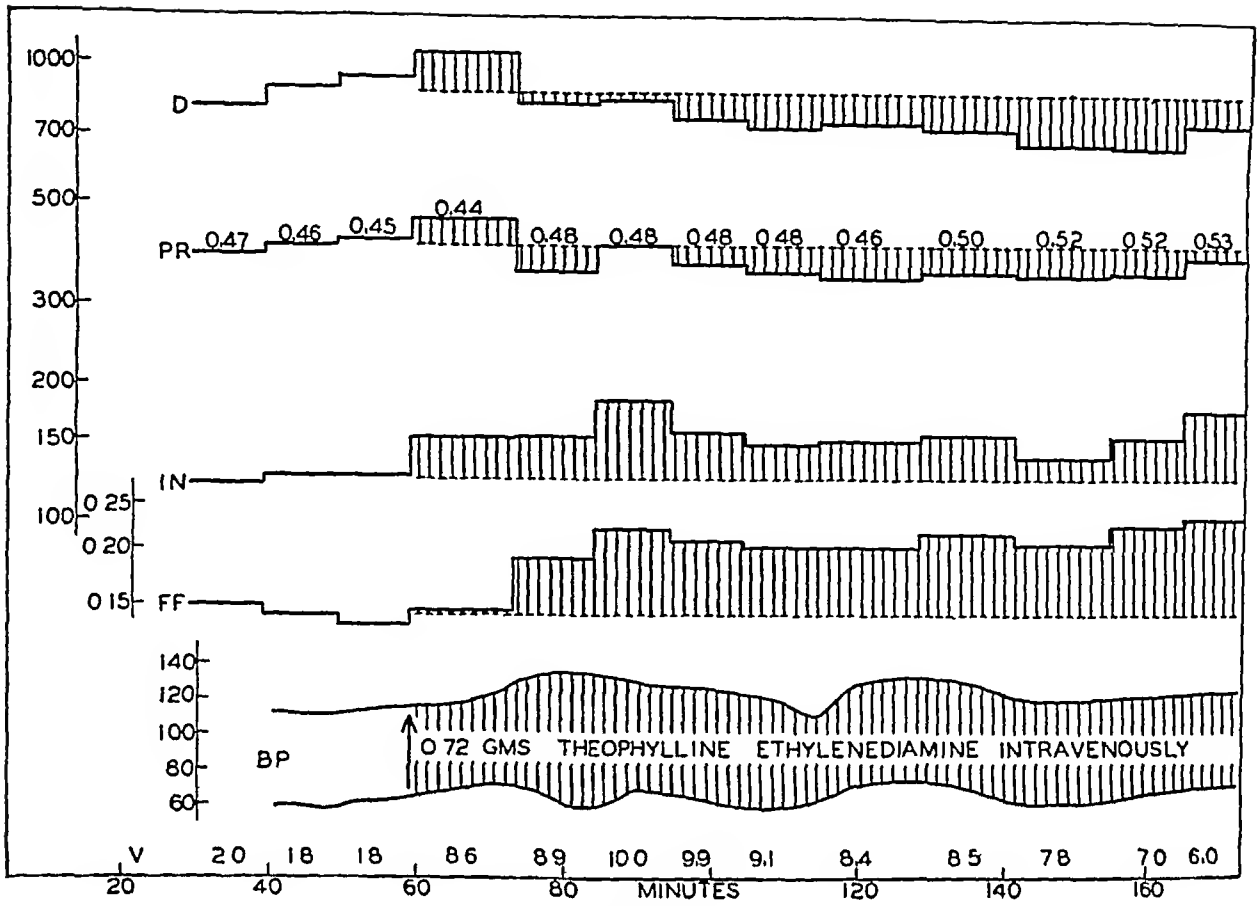


FIG 5
Data on Subject B L

held to have only a remote bearing upon the normal organism By the oncometer it is impossible to distinguish enlargement of the kidney caused by vasodilatation, diuresis, or glomerular distension (2, 21, 25) Gremels (8) occasionally, and Verney and Winton (32) almost invariably, obtained an increase in the perfusion rate of the heart-lung-kidney after the addition of caffeine, theophylline, etc It is known, however, that the heart-lung-kidney is definitely unphysiological, normal, concentrated urine cannot be obtained, and at a constant perfusion pressure the blood flow may increase spontaneously 100 per cent in consequence of changes in the tonus of the renal vessels (7) Shed blood rapidly develops powerful vasoconstrictor power (12) these "vasotonins" are supposed to be removed by perfusion through the lungs (3, 11, 29), yet when a freshly isolated kidney is perfused by the animal's own circulation vasotonic substances are given off into the systemic blood (5) One may on the above evidence question the basal vasomotor tone and the responses to drugs of the heart-lung-kidney The same criticism applies to the pump-kidney (17) Using a thermostromuhr applied to the renal artery of anesthetized dogs, Janssen and Rein (14) obtained renal hyperemia after 3 mgm per kgm of caffeine *per os,* but the details of their experiments showing how long the hyperemia lasted are not available, and the observation needs confirmation on unanesthetized and untraumatized animals The only available observations on unanesthetized animals are those of Walker, Schmidt, Elsom, and Johnston (33), which were made by a thermostromuhr applied to the abdominal aorta of rabbits with suitable ligations to restrict the blood flow to one kidney The observations were made a few hours after operation These investigators found that theophylline injected intravenously in doses of 12 mgm produced, in seven out of ten experiments, an increased blood flow which was, however, transient, lasting on the average only 9

minutes Thereafter, the blood flow returned to or below its former level In two of the four experiments which these investigations report graphically the blood flow was at a reduced level after the administration of the drug But again we call attention to the uncertainty of transferring observations on glomerular hemodynamics from the rabbit to the dog or man.

From the above, it will be seen that there is no certain evidence on which to conclude that xanthine derivatives in therapeutic doses induce renal hyperemia in the normal animal On the contrary, we conclude from a study of the diodrast clearance that theophylline and caffeine consistently reduce the blood flow through the normal human kidney In the observations recorded in Figure 5, 0.72 gram of theophylline ethylenediamine given intravenously reduced the diodrast clearance from its mean control value of 875 cc. per minute to a minimal value of 670 cc. per minute. Simultaneously, the filtration rate was increased from 123 to a value above 150 cc., and the filtration fraction increased from 14.2 per cent to a value above 20 per cent After the administration of theophyl line the phenol red/diodrast clearance ratio, if it changed significantly, increased, as is to be expected during a period of renal ischemia. From this fact we feel confident that the reduction of the diodrast clearance is not due to an interfering action of the drug upon the excretory activity of the tubules, but to an actual reduction in plasma flow

We have examined the action of theophylline in four other instances, and the action of caffeine sodium benzoate in two instances Theophylline ethylenediamine was given intravenously in doses of 0.96, 1 05 and 1 2 grams, and once by constant intravenous infusion at the rate of 17 mgm per minute. Caffeine sodium benzoate was given subcutaneously in a dose of 450 mgm , and a half hour later an additional dose of 450 mgm was injected intravenously On another occasion 300 mgm , which is essentially a minimal effective dose so far as cerebral effects are concerned were given orally In every case, the results were qualitatively the same as those shown in Figure 5, except that with larger doses the reduction in plasma flow and increase in filtration fraction were much more marked The largest doses of theophylline decreased the plasma flow from 450 cc. to a minimum of 255 cc., and raised the filtration fraction from 17 5 per cent to a maximum of 35 per cent, 300 mgm of caffeine decreased the plasma flow from 630 to 540 cc. per minute and increased the filtration fraction from 18 to 22 per cent.

The xanthine derivatives increase the cardiac output and decrease the peripheral resistance (13, 15, 30), showing that they dilate some arterioles in normal man , it may be that they dilate the afferent glomerular arterioles and thus contribute to the elevation of filtration pressure effected by efferent constriction Thus would explain the circumstance that the filtration rate, which is relatively unaffected by adrenin, may in some instances be markedly increased by theophylline and caffeine, as in Figure 5 , and, if afferent dilatation preceded efferent constriction in time, it would explain the fact that in three instances, including the subject reported in Figure 5, the renal plasma flow was increased during the first period after the administration of the drug But if afferent dilatation occurs, it is overshadowed by efferent constriction with, in the mean, a reduction in renal blood flow

Whether the xanthine derivatives act locally upon the renal vessels or through the central nervous system is not known

The diuretic action of these compounds is notoriously uncertain In the observations recorded in Figure 5 the urine flow rose from 1 8 cc. to a maximum of 9 9 cc. Here, and in some other instances, the increased urine flow is correlated with an increased filtration rate, but evidence has been presented that this is not the essential mechanism of xanthine diuresis (25) In interpreting this diuresis the possible influence of these substances upon the central nervous system and the nervous control of the pituitary gland must not be overlooked

Typhoid vaccine pyrexia

(Figure 6 Subject D W , 1 63 sq m , 58 per cent plasma.) We have noticed during the course of the reaction elicited by pyrogenic infusions that the renal plasma flow may rise to values considerably above normal Since pyrexial reactions induced by the intravenous injection of vaccines of the typhoid group are widely used in the therapy of chorea, thrombo-angiitis ob her diseases, we present in Figure

rexial reaction induced by the intravenous administration of typhoid vaccine

The renal blood flow appears to be controlled predominantly by the efferent glomerular arterioles, these arterioles being normally partially constricted Since, with such efferent control, an increase or decrease in renal blood flow is accompanied by an inverse change in filtration pressure, the filtration fraction varies inversely to, and the filtration rate tends to be independent of, the renal blood flow

BIBLIOGRAPHY

1 Cow, D, Einige Studien über Diurese. Arch f exper Path u. Pharmakol, 1912, 69, 393
2 Cushny, A R., The Secretion of the Urine. Longmans, Green & Co, New York, 1917
3 Eichholtz, F, and Verney, E. B, On some conditions affecting the perfusion of isolated mammalian organs J Physiol., 1924, 59, 340
4 Ellinger, P, Die Absonderung des Harns unter verschiedenen Bedingungen einschliesslich ihrer nervösen Beeinflussung und der Pharmakologie und Toxikologie der Niere Vol. IV Handb d. Norm. u Path Physiol., 1929, 308–450
5 Enger, R., and Gerstner, H, Der Einfluss der Niere auf den Blutdruck nach ihrer völligen Lösung aus dem Gewebszusammenhang des Organismus Ztschr f d. ges exper Med, 1938, 102, 413
6 Forster, R. P, Xylose, inulin and creatinine clearance in the normal frog Proc. Soc. Exper Biol and Med, 1938, 38, 258
7 Gelfan, S, and Visscher, M B, Changes in renal blood flow in relation to changes in pressure in urine formation Proc. Soc. Exper Biol and Med, 1937, 36, 27
8 Gremels, H, Über die Wirkung einiger Diuretika am Starlingschen Herz-Lungen-Nierenpräparat Arch f exper Path u Pharmakol., 1928, 130, 61
9 Grollman, A, Variations in the cardiac output of man V The cardiac output of man during the malaise and pyrexia following the injection of typhoid vaccine. J Clin Invest., 1929, 8, 25
10 Hartmann, H, Ørskov, S L, and Rein, H, Die Gefässreaktionen der Niere im Verlaufe allgemeiner Kreislauf-Regulationsvorgänge Pflüg Arch. f d. ges Physiol, 1937, 238, 239
11 Hemingway, A, Some observations on the perfusion of the isolated kidney by a pump J Physiol., 1931, 71, 201
12 Herrick, J, and Markowitz, J, The toxic effects of defibrinated blood when perfused through the isolated mammalian heart. Am J Physiol, 1929, 88, 698
13 Hoen, E., and Neuthard, A, Pharmakologische Untersuchungen über das Minutenvolumen des normalen menschlichen Herzens II Der Einfluss von Cardiazol, Coramin und Coffein.- Natr salicyl Arch f exper Path. u Pharmakol, 1937, 185, 302–308
14 Janssen und Rein, Über die Zirkulation und Wärmebildung der Niere unter Einfluss von Giften. Arch f exper Path u. Pharmakol., 128, Verhandl d deutsch Pharm Gesellsch., 1928, 107
15 Neuthard, A., and Hoen, E., Pharmakologische Untersuchungen über das Minutenvolumen des normalen menschlichen Herzens I Der Einfluss von Coffein, Theobromin, Theophyllin, Kaffee und Kaffee Hag Arch. f exper Path u. Pharmakol, 1937, 185, 293
16 Opitz, E., and Smyth, D H, Nierendurchblutung bei Reizung des Carotis-Sinus Pflügers Arch f d. ges Physiol., 1937, 238, 633
17 Ozaki, M, Pharmakologie der Nierengefässe. Arch f exper Path u Pharmakol, 1927, 123, 305
18 Pitts, R. F, The excretion of phenol red by the chicken. J Cell and Comp Physiol, 1938, 11, 99
19 Ranson, S W, Jr, Fever induced by the intravenous injection of typhoid-paratyphoid vaccine. Arch. Int. Med, 1938, 61, 285
20 Rein, H, and Rössler, R., Die Abhängigkeit der vasomotorischen Blutdruckregulation bei akuten Blutverlusten von den thermoregulatorischen Blutverschiebungen im Gesamtkreislaufe. Ztschr f Biol, 1929, 89, 237
21 Richards, A N, and Plant, O H, Urine formation in the perfused kidney The influence of adrenalin on the volume of the perfused kidney Am J Physiol, 1922, 59, 184
22 Richards, A N, and Plant, O H, The action of minute doses of adrenalin and pituitrin on the kidney Am J Physiol, 1922, 59, 191
23 Rydin, H, and Verney, E. B, The inhibition of water-diuresis by emotional stress and by muscular exercise. Quart. J Exper Physiol, 1938, 27, 343
24 Shannon, J A., The excretion of phenol red by the dog Am J Physiol, 1935, 113, 602
25 Smith, H W, The Physiology of the Kidney Oxford University Press, New York, 1937
26 Smith, H W, Goldring, W, and Chasis, H, The measurement of the tubular excretory mass, effective blood flow and filtration rate in the normal human kidney J Clin Invest., 1938, 17, 263
27 Smith, W W, and Ranges, H, Renal clearances of iopax, neoiopax and skiodan in man Am. J Physiol, 1938, 123, 720
28 Springorum, P W, and Centenera, D, Die verschiedene Beteiligung beider Nieren an Diureseänderungen und vasomotorischen Reaktionen. Pflüg Arch f d ges Physiol, 1937, 239, 440
29 Starling, E. H, and Verney, E. B, The secretion of urine as studied on the isolated kidney Proc. Roy Soc., s B, 1925, 97, 321
30 Starr, I, Gamble, C J, Margolies, A, Donal J S., Jr, Joseph, N., and Eagle, E, A clinical study of

the action of 10 commonly used drugs on cardiac output, work and size, on respiration, on metabolic rate and on the electrocardiogram. J Clin. Invest., 1937, 16, 799

31. Uma, K., Arterieller Druck und Nierendurchblutung Pflüg Arch f d. ges. Physiol., 1935, 235, 515
32. Verney, E. B., and Winton, F R., The action of caffeine on the isolated kidney of the dog J Physiol., 1930 69, 153
33 Walker, A. M, Schmidt., C F., Elsom K. A., and Johnston, C. G, Renal blood flow of unanesthetized rabbits and dogs in diuresis and antidiuresis. Am. J Physiol., 1937 118 95
34 Wilkins, R. W., Haynes F W., and Weiss, S., The rôle of the venous system in circulatory collapse induced by sodium nitrite. J Clin. Invest., 1937, 16, 85
35 Winton, F R., The glomerular pressure in the isolated mammalian kidney J Physiol., 1931, 72, 361
36 Winton, F R., The control of the glomerular pressure by vascular changes within the isolated mammalian kidney, demonstrated by the actions of adrenaline. J Physiol., 1931, 73, 151

A STUDY OF SOME OF THE PHYSIOLOGICAL EFFECTS OF SULFANILAMIDE II METHEMOGLOBIN FORMATION AND ITS CONTROL

By ALEXIS F HARTMANN, ANNE M. PERLEY, AND HENRY L. BARNETT

(From the Department of Pediatrics Washington University School of Medicine and the St Louis Children's Hospital St Louis)

(Received for publication May 6, 1938)

One of the most commonly observed toxic effects of the administration of moderately large doses of sulfanilamide is the development of methemoglobinemia with resulting cyanosis and reduction in the oxygen carrying capacity of the blood Sulphemoglobinemia also has been reported, and Marshall and Walzl (1) have postulated the presence of a black oxidation product of sulfanilamide which stains the red blood cells

The chemical nature of methemoglobin and the conditions necessary for its formation are discussed in some detail by Peters and Van Slyke (2) A wide variety of agents may promote the formation of methemoglobin from reduced hemoglobin but the chemical reaction taking place presumably always involves the oxidation of the ferrous iron, $\ddot{F}e$, in ordinary hemoglobin to the ferric iron, $\ddot{F}e$, in methemoglobin The exact manner in which sulfanilamide, or a by product of sulfanilamide, promotes such an oxidation is still unknown It is stated by Archer and Discombe (3) that it is highly probable that all drugs containing the group $C_6H_5N <$ are capable of causing methemoglobinemia and of facilitating the production of sulphemoglobin In regard to the formation of the latter, Harrop and Waterfield (4) have shown as pointed out particularly by Paton and Eaton (5), that while various aromatic compounds promote methemoglobinemia, when these compounds are given along with sulphur the result may be sulphemoglobinemia The latter investigators feel that methemoglobin is the true toxic result of a large dose of sulfanilamide, or possibly of quite a moderate dose in an unusually susceptible person, and sulphemoglobin formation takes place only when sulphur compounds are available, as is the case when they are present in the bowel in unusually large amounts Methemoglobinemia, therefore, can be considered the more direct result of sulfanilamide administration, and in the cases which we are to report, by far the most important cause of the cyanosis

The formation of methemoglobin is reversible, and the reconversion of methemoglobin to hemoglobin in the body takes place slowly, so that when the factor which is promoting excessive methemoglobin formation is withdrawn the methemoglobinemia gradually disappears However, in patients receiving sulfanilamide, the factor contributing to methemoglobin production is present certainly as long as the drug is being given As will be pointed out later, continued rapid reconversion of methemoglobin to hemoglobin may be desirable This has been accomplished by the use of methylene blue.

Hauschild (6) in June 1937 reported upon the effectiveness of Katalysin (thionin) as an antidote for methemoglobinemia produced in animals by the injection of sodium nitrite, analine, nitrobenzol, and para aminophenol In investigations on cats and rabbits, he showed by quantitative estimation that after the methemoglobin concentration had risen to 40 to 50 per cent of the total pigment, the intravenous injection of thionin caused nearly all of it to be reconverted to hemoglobin within ten minutes He feels that the action takes place by means of the reversible oxidation-reduction system of thionin leucothionin, the system of hemoglobin methemoglobin being shifted in favor of hemoglobin In an earlier investigation (7), he reported the effectiveness of both thionin and methylene blue (tetramethyl thionin HCl) as an antidote in methemoglobin poisoning, but at that time was in doubt as to the nature of the mechanism involved

Wendel (8) in October 1937 proposed the use of methylene blue in the treatment of globinemia resulting from the sulfanilamide He was unable to p

moglobinemia in dogs and rabbits by the administration of sulfanilamide, even in large doses, but observed that methylene blue given intravenously to animals poisoned with sodium nitrite greatly increased the rate of reconversion of methemoglobin to hemoglobin He reported upon two of our children who were being treated with sulfanilamide, and in whom a single intravenous injection of 1 mgm of methylene blue per kilogram of body weight reduced the methemoglobin from 20 to 18 per cent of the total pigment, respectively, to less than 3 per cent in 30 minutes He pointed out that Williams and Challis in 1933 reported that methylene blue was an effective antidote for para-brom-analine poisoning, and that shortly afterwards, Steele and Spink used methylene blue in a case of analine poisoning and one of acetanilid poisoning with what they considered dramatic recoveries Both groups of workers stated that the methemoglobinemia shown by their patients before the administration of methylene blue rapidly disappeared

METHODS

In the studies to be reported, direct estimation of the amount of methemoglobin in the blood was made by a simple spectroscopic method devised by Wendel (personal communication) In this procedure, by properly diluting the unknown sample, the intensity of the methemoglobin absorption band is made to equal that of a standard which is prepared from a sample of the same blood by completely converting the hemoglobin to methemoglobin by the addition of potassium ferricyanide and diluting to a convenient working concentration The amount of methemoglobin is then expressed as per cent of the total pigment In a number of instances the amount of non-oxygen carrying hemoglobin was determined indirectly by the difference between the total hemoglobin present, as estimated by the acid hematin method of Sahli, and the functional hemoglobin as determined by the oxygen capacity (9), of the same sample of blood The correlation between these two independent methods of determining the amount of hemoglobin incapable of combining with oxygen, as can be seen from Table I, is reasonably close, and the possible inaccuracies inherent in two of the three methods employed are great enough to account for most of the discrepancies in the results These data are included to show that, if spectroscopic examination of the blood of patients suspected of having methemoglobinemia from sulfanilamide therapy is not possible, determination of the oxygen capacity and of total pigment of the blood provides a reasonably reliable substitute, which is confirmatory of an opinion expressed in an earlier report of Basman and Perley (10)

TABLE I

Correlation between concentrations of methemoglobin and per cent of non-oxygen carrying hemoglobin

Case	Oxygen carrying hemoglobin (oxygen capacity ÷ 1.34)	Total hemoglobin pigment (Sahli)	Amount of total hemoglobin pigment present as non-oxygen carrying hemoglobin*	Amount of total hemoglobin pigment present as methemoglobin†
	grams hemoglobin per 100 cc.	*grams hemoglobin per 100 cc.*	per cent	per cent
J L.	10 4	11.2	7	3
	9.3	11.2	17	12
	10 0	11.2	11	10
	9.9	11 0	10	11
	9.5	10.5	9	17
	8.8	10.5	16	19
W A.	6 9	8.0	14	14
M. R.	9 7	13.3	26	33
	11.2	13.3	16	12
	11 0	13.3	17	16
	11 4	12 0	5	4
D C	9 9	11.5	14	14
W H.	11 1	13.5	18	23
	12.5	13.8	9	6
	10 1	12 6	20	23
	10 1	13 0	22	24
	11.9	13 0	8	10
	8 6	10 0	14	13
	9 0	10 0	10	9
B G	7 9	8.8	10	5
	7.5	8.8	15	7
	9 7	11.5	16	10
	9.3	10 4	11	13
	9 6	10 7	10	12
G G	9.9	11 7	15	21
J L	7 0	8.8	20	26

* As calculated from the equation

$$\frac{\text{Sahli hemoglobin} - (\text{oxygen capacity} \div 1.34)}{\text{Sahli hemoglobin}} \times 100$$

† As determined directly by the spectroscopic method of Wendel

The sulfanilamide concentration of the blood was determined by the method of Marshall (11)

Methemoglobin accumulation

Frequency of methemoglobin accumulation in human subjects following sulfanilamide administration In almost every patient treated with sulfanilamide, in doses over 0 1 gram per kgm per 24 hours, we have observed some degree of cyanosis This cyanosis is of a characteristic shade, and, after some experience, the observer can usually differentiate it from the usual type of cyanosis caused only by reduced hemoglobin

Coincidently, in every case in which cyanosis was observed and in which the blood of the patient was examined spectroscopically, we were able to detect the absorption band characteristic of methemoglobin.

Rate of methemoglobin accumulation following sulfanilamide administration There is a marked individual variation in the rate at which methemoglobin accumulates following the administration of sulfanilamide, but the rate of accumulation in general depends upon the dose. With an initial large dose of sulfanilamide, equivalent to 0.15 to 0.2 gram per kgm., given either orally or subcutaneously, clinically recognizable methemoglobin cyanosis usually becomes manifest in from 2 to 5 hours. In most cases this corresponds to a blood methemoglobin concentration of at least 10 per cent of the total pigment. In patients receiving 0.1 gram per kgm. or less per 24 hours, clinical cyanosis, if it occurs at all, may not become evident until 2 or 3 days after the administration has been started. Table II shows the rate of accumulation in several patients receiving different amounts of sulfanilamide.

Relation of the degree of methemoglobin accumulation to the sulfanilamide concentration of the blood While there seems to be some correlation between the sulfanilamide concentration of the blood and the degree of methemoglobinemia, as is seen from Figure 1, the degree of methemoglobin accumulation which occurs following sulfanilamide administration seems to depend more upon an individual characteristic than upon the sulfanilamide concentration. We have gained the clinical impression that the greater the toxicity of the patient and the more marked the

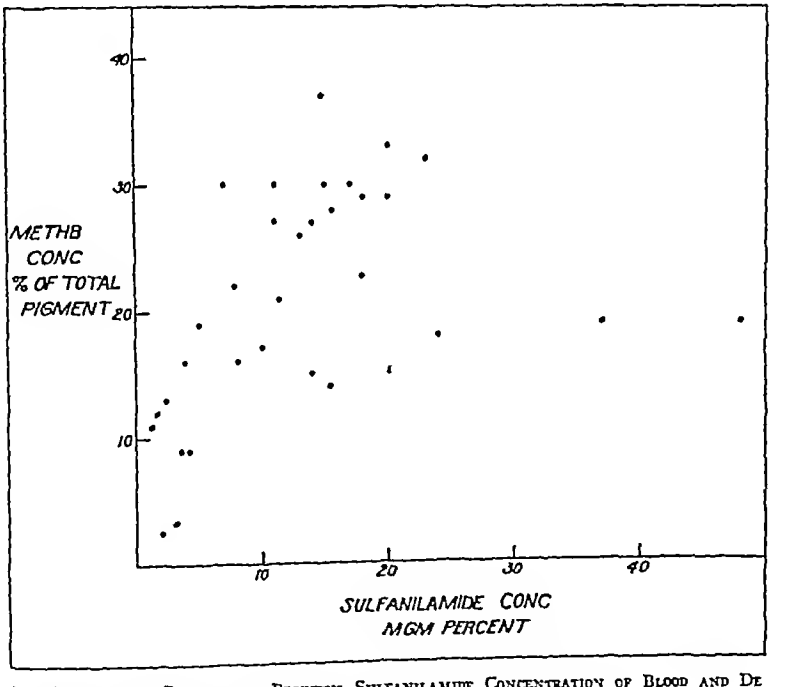


FIG. 1. GENERAL CORRELATION BETWEEN SULFANILAMIDE CONCENTRATION OF BLOOD AND DEGREE OF METHEMOGLOBINEMIA

Each dot represents the sulfanilamide and methemoglobin concentrations in of blood.

TABLE II

Rate of accumulation of methemoglobin following sulfanilamide administration

Case	Sulfanilamide administration	Time after starting sulfanilamide	Methemoglobin concentration	Sulfanilamide concentration
			per cent of total pigment	*mgm. per cent*
J L.	0.2 gram per kgm. as initial dose, followed by 0.2 gram per kgm. per 24 hours in 6 divided doses after 48 hours increased to 0 4 gram per kgm., and after 72 hours to 0 6 gram per kgm.	45 minutes	3	
		5 hours	12	21 7
		71 hours	17	38.8
		84 hours	19	48.2
J L.	0.2 gram per kgm. as initial dose, followed by 0.2 gram per kgm. per 24 hours in 6 divided doses.	6½ hours	12	21 7
		12 hours	10	17 6
		20 hours	11	17 9
H.B	0.2 gram per kgm. in 6 divided doses.	14 hours	15	14.0
		26 hours	27	17 0
D G	0 1 gram per kgm. per 24 hours in 6 divided doses.	1 day	6	7 7
		2 days	9	10 6
		4 days	15	14.1
		6 days	13	8.9
L. S.	0.1 gram per kgm. per 24 hours in 6 divided doses.	12 hours	3	2.2
		2 days	5	3 4
		4 days	9	
		5 days	9	3 8
		6 days	9	4.3

acute inflammatory process, the greater the tendency for methemoglobin accumulation Four of the five patients in whom methemoglobin concentrations were above 30 per cent of the total pigment, with sulfanilamide concentrations of the blood below 20 mgm per cent, were extremely ill, having severe infections and high fevers In a given individual, however, there does seem to be better correlation between sulfanilamide and methemoglobin concentrations, as can be seen from Table II and Figure 2

Rate of reconversion of methemoglobin to hemoglobin

After withdrawal of sulfanilamide The methemoglobin accumulating as a result of the administration of sulfanilamide is slowly reconverted to hemoglobin following withdrawal of the drug The fall roughly parallels the decrease in the sulfanilamide concentration of the blood, requiring usually from 24 to 72 hours, depending upon the concentrations of the two reached prior to the withdrawal of sulfanilamide (Figure 2)

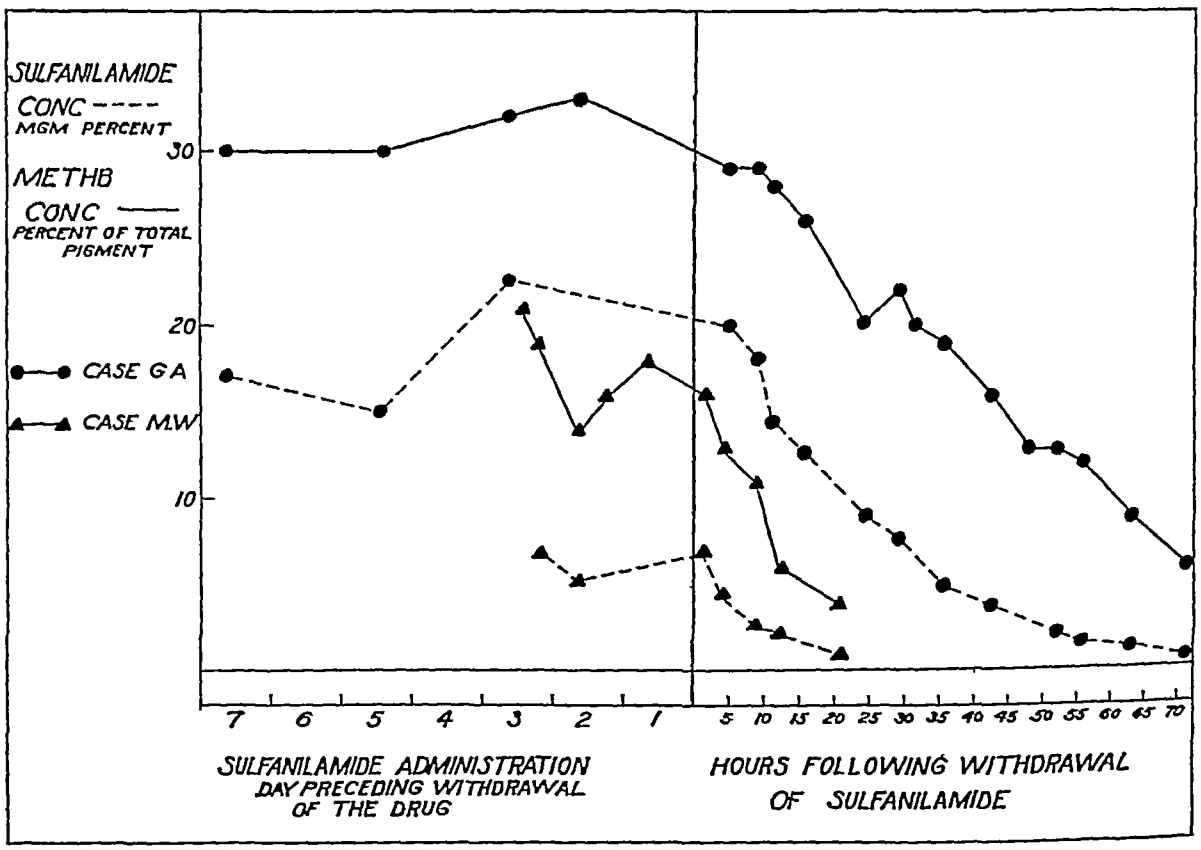


FIG 2. CORRELATION BETWEEN SULFANILAMIDE CONCENTRATION OF BLOOD AND DEGREE OF METHEMOGLOBINEMIA IN INDIVIDUAL CASES, SHOWING PARTICULARLY THE RATE OF DISAPPEARANCE OF METHEMOGLOBIN AFTER THE DISCONTINUATION OF SULFANILAMIDE.

Following the intravenous injection of methylene blue with continued sulfanilamide administration The use of methylene blue for the control of severe methemoglobinemia was begun by us at the suggestion of Wendel At first, the dye was administered intravenously as a one per cent solution, the dosage being from 1 to 1 5 mgm. of methylene blue per kgm. of body weight. The effectiveness of this dosage in reducing the methemoglobinemia is shown in Figure 3 The decrease or complete disappearance of cyanosis observed simultaneously with the fall in methemoglobin in the blood is quite spectacular, especially when the original cyanosis was intense The effect is practically complete within 30 minutes Figure 3 also demonstrates that following its reduction, methemoglobin reaccumulates at a rapid rate

After the oral administration of methylene blue with continued administration of sulfanilamide Again at the suggestion of Wendel, we undertook to control the accumulation of methemoglobin by the oral administration of methylene blue. The dose usually employed was one or two grains (65 or 130 mgm) every 4 hours, depending upon both the size of the patient and the dose of sulfanilamide given Figures 4 to 9 demonstrate the effectiveness of this method of administration

PROTOCOLS

Case 1 Donald B., a 4½ year-old white boy weighing 14.5 kgm., was admitted to the hospital with bilateral mastoiditis which required operation After the patient had received approximately 0.2 gram of sulfanilamide per kgm. per 24 hours for 6 days, the methemoglobin concentration was found to be 16 per cent of the total pigment, and the blood sulfanilamide 7.9 mgm. per cent. With the same dose of sulfanilamide continued, the patient was given one gram of methylene blue five times daily at 4 hour intervals Ten hours after the administration of the first dose of methylene blue there was noted a definite fall in the methemoglobin concentration to 4 per cent. A low level was maintained throughout the following 6 days of observation. The blood sulfanilamide concentration ranged between 7 4 and 10.9 mgm. per cent during this period.

In this small child it is apparent that with the administration of 0.2 gram of sulfanilamide per kgm. of body weight per 24 hours, there was considerable accumulation of methemoglobin which however, was well controlled by the administration of only one gram of methylene blue 5 times a day

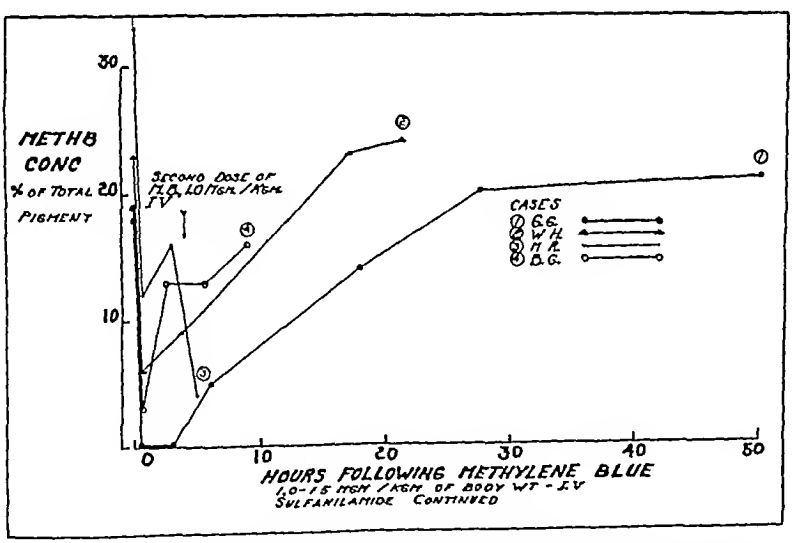


FIG. 3 RAPID REDUCTION OF METHEMOGLOBINEMIA FOLLOWING THE INTRAVENOUS ADMINISTRATION OF METHYLENE BLUE, AND RATE OF METHEMOGLOBIN REACCUMULATION

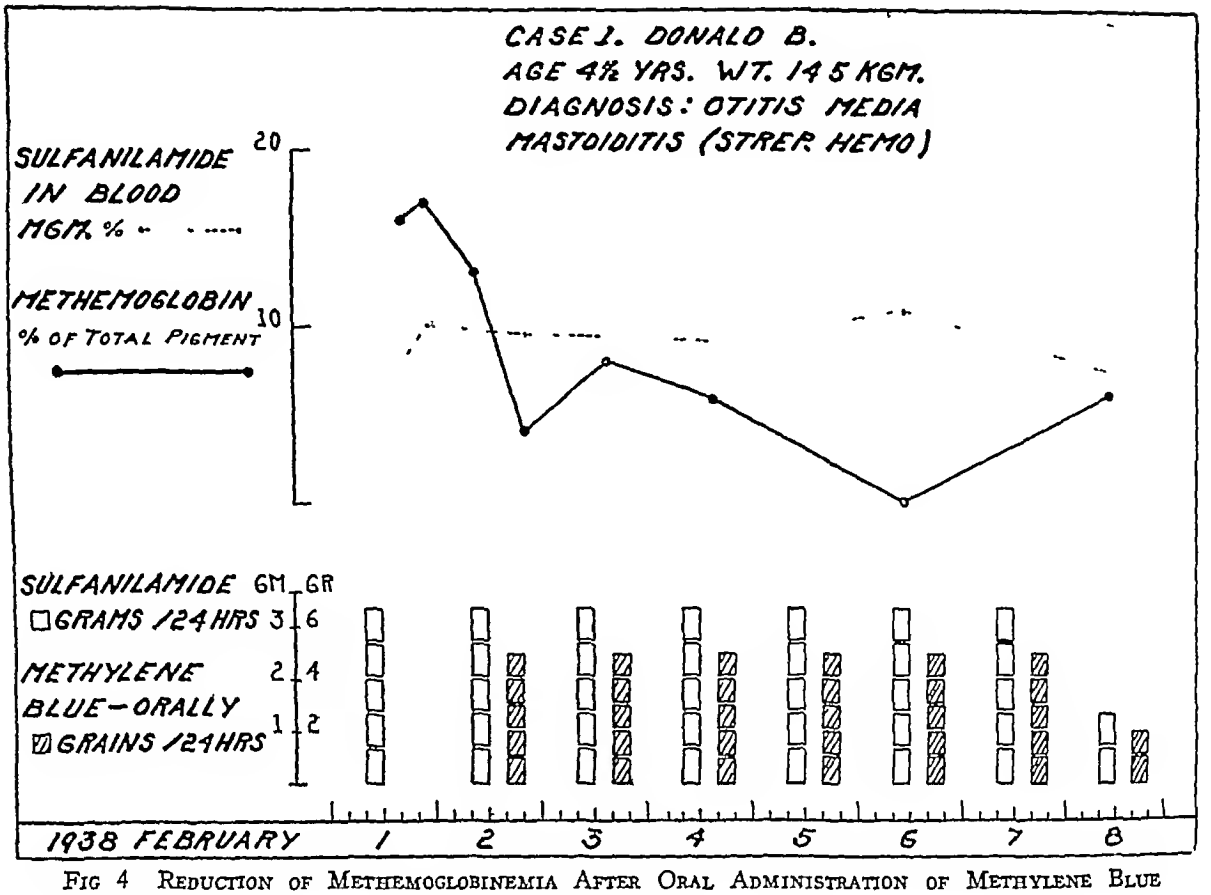


FIG 4 REDUCTION OF METHEMOGLOBINEMIA AFTER ORAL ADMINISTRATION OF METHYLENE BLUE

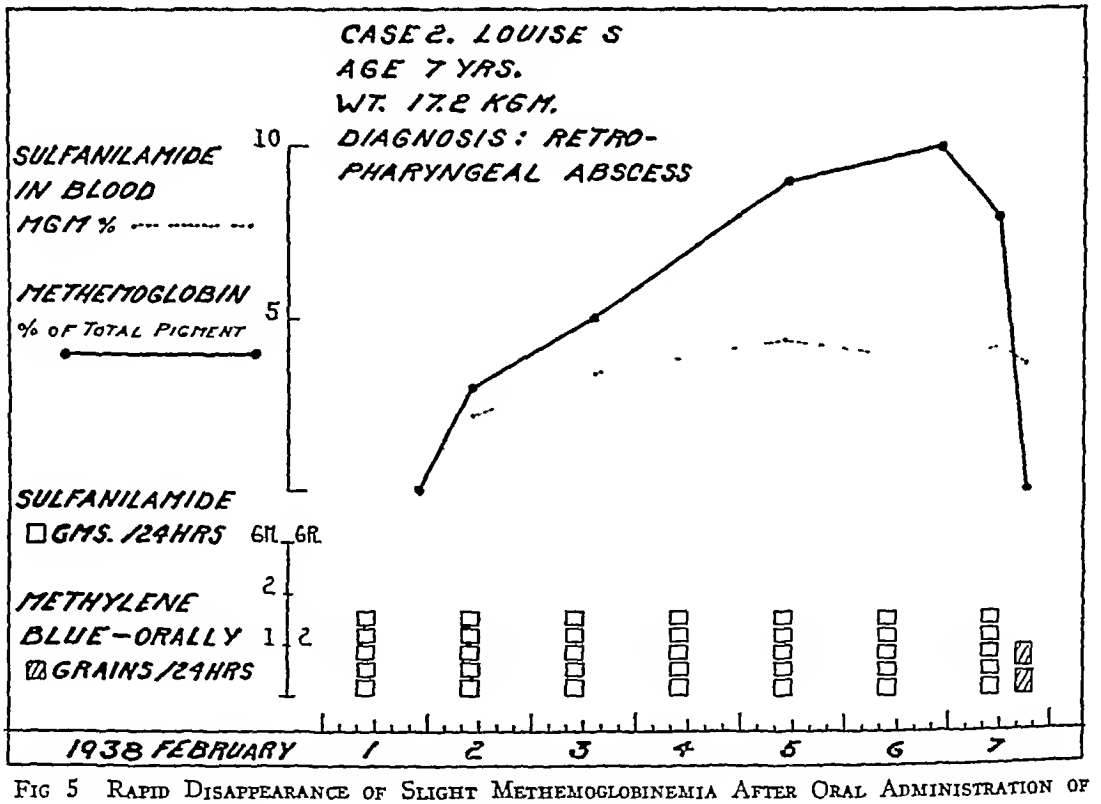


FIG 5 RAPID DISAPPEARANCE OF SLIGHT METHEMOGLOBINEMIA AFTER ORAL ADMINISTRATION OF METHYLENE BLUE

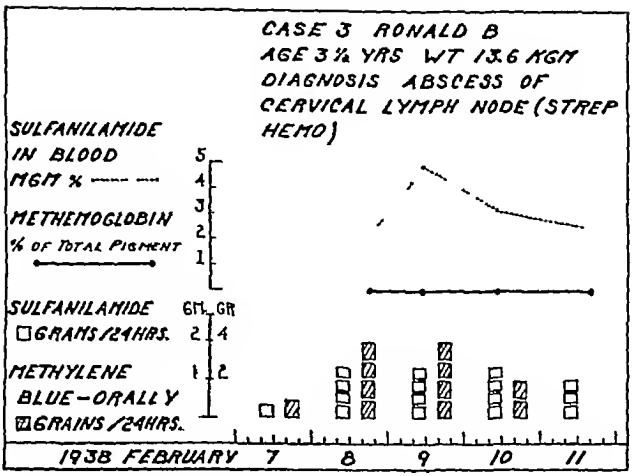


FIG. 6 PREVENTION OF ACCUMULATION OF METHEMOGLOBIN BY ORAL ADMINISTRATION OF METHYLENE BLUE

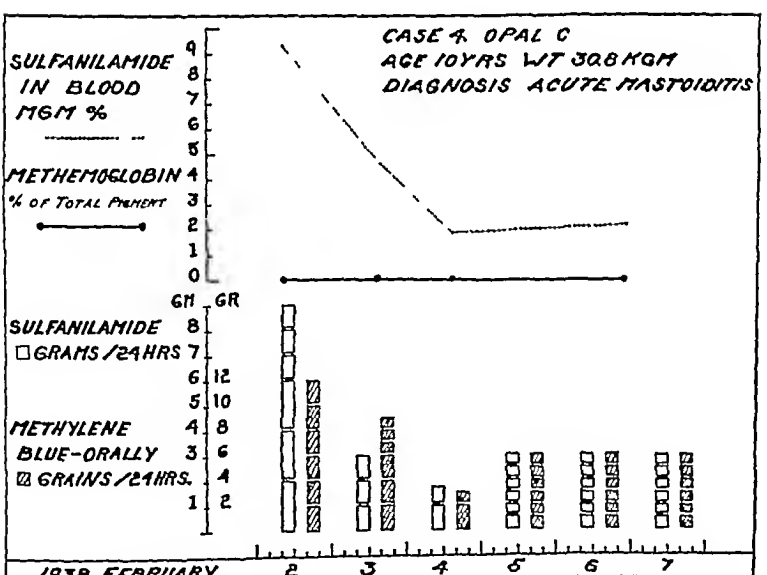


FIG. 7 EFFECTIVENESS OF ORAL ADMINISTRATION OF METHYLENE BLUE IN PREVENTING ACCUMULATION OF METHEMOGLOBIN FOLLOWING LARGE INITIAL DOSE OF SULFANILAMIDE

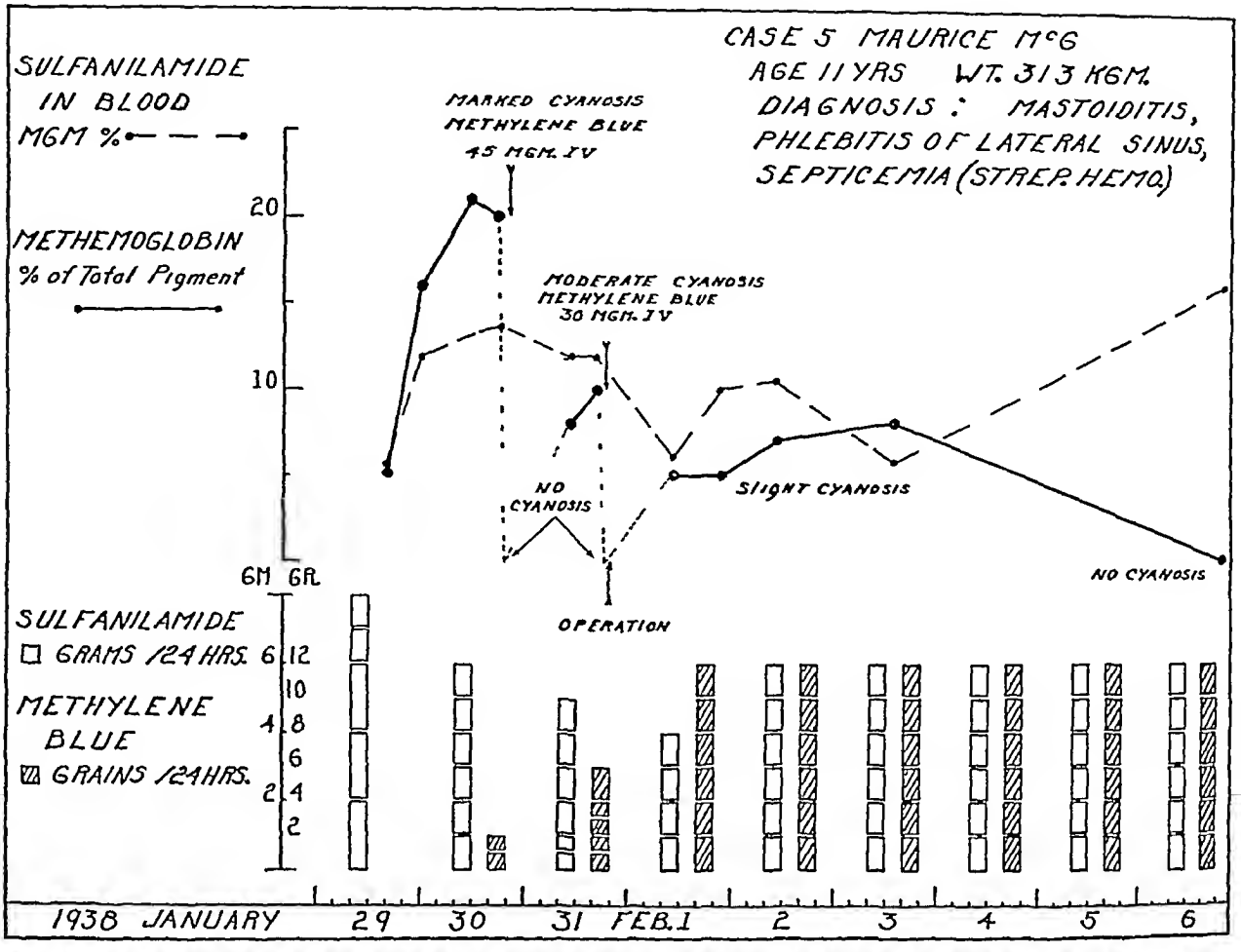


FIG 8 REDUCTION OF METHEMOGLOBINEMIA BY INTRAVENOUS ADMINISTRATION OF METHYLENE BLUE. RECURRENCE WITH INADEQUATE ORAL ADMINISTRATION, AND SUBSEQUENT BETTER CONTROL BY INCREASED DOSE

Case 2 Louise S, a 7-year-old white girl, weighing 17.2 kgm., was admittted to the hospital with a diagnosis of retropharyngeal abscess She was given 0 1 gram of sulfanilamide per kgm per 24 hours, and methemoglobin was allowed to accumulate for a period of 6 days, during which time its concentration slowly rose to a level of 10 per cent of the total pigment On the seventh day, the administration of one grain of methylene blue every 4 hours, along with the sulfanilamide, was started Only one subsequent determination of methemoglobin was made, 5 hours after the administration of methylene blue was begun, and at this time no methemoglobin band was detected

It is apparent that this patient developed a relatively small amount of methemoglobin with the administration of 0 1 gram of sulfanilamide per kgm. per 24 hours, and 2 doses only of methylene blue, one grain each, were sufficient to cause disappearance of the methemoglobin band.

Case 3 Ronald B, a 3½-year-old white boy, weighing 13 6 kgm., was admitted to the hospital because of postscarlatinal cervical adenitis The administration of approximately 0 1 gram of sulfanilamide per kgm per 24 hours and one grain of methylene blue every 6 hours was begun immediately, and on 3 subsequent days examination of the blood revealed no methemoglobin band, with the sulfanilamide concentration of the blood ranging from 2.2 to 4 9 mgm per cent. The methylene blue was discontinued on the fourth day, and the patient was discharged 24 hours later, by which time no methemoglobin had yet accumulated

This case demonstrates the prevention of the accumulation of any detectable amount of methemoglobin by the administration of one grain of methylene blue every 6 hours when 0 1 gram of sulfanilamide per kgm. per 24 hours was being given

Case 4 Opal C, a 10-year-old white girl, weighing 30 8 kgm, was admitted to the hospital on February 1, 1938, acutely ill with mastoiditis and associated *cellulitis* The following day, February 2, 1938, she was given an initial dose of sulfanilamide, 0.2 gram per kgm. in 3 divided doses, between 10 00 a.m and 12 00 noon. With each dose of sulfanilamide she received 2 grains of methylene blue. Following this initial dose, she was

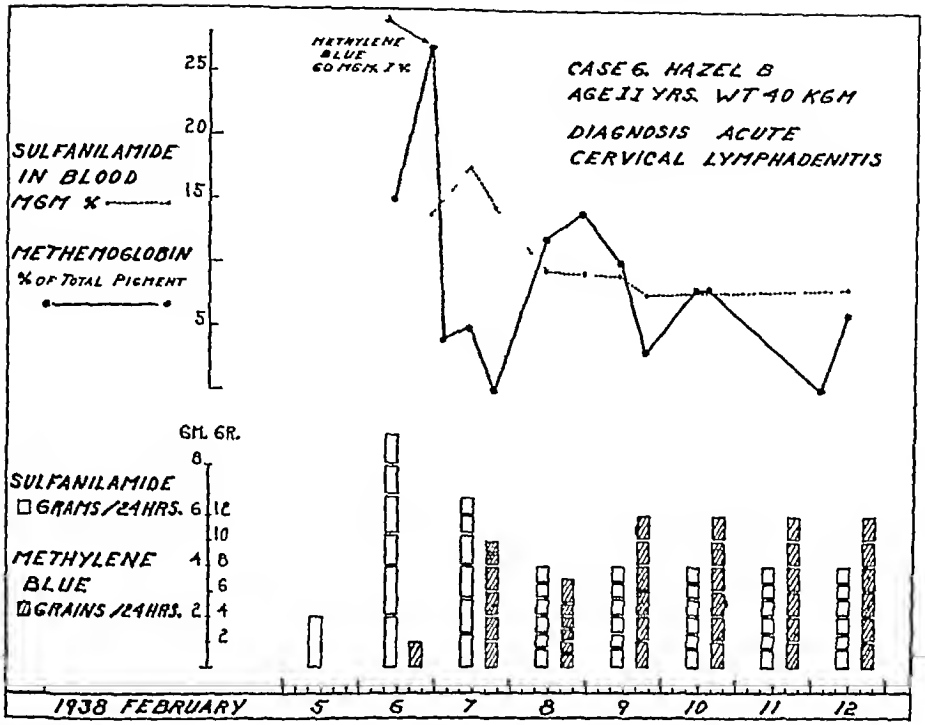


FIG. 9 MARKED METHEMOGLOBINEMIA ELIMINATED BY INTRAVENOUS INJECTION OF METHYLENE BLUE AND CONTROLLED BY ORAL ADMINISTRATION REACCUMULATION OF METHEMOGLOBIN WITH DOSES OF SULFANILAMIDE AND METHYLENE BLUE PROPORTIONATELY REDUCED, LATER CONTROL WITH INCREASED METHYLENE BLUE.

given 0.2 gram of sulfanilamide per kgm. per 24 hours and 2 grains of methylene blue every 4 hours through 12:00 noon on February 3 1938 at which time the sulfanilamide was discontinued because of vomiting which was thought to be caused by the administration of the drug However, because a mastoidectomy was to be performed the next morning the methylene blue was continued in one half its former dose to prevent any accumulation of methemoglobin. One dose of sulfanilamide and one of methylene blue were then given 4 hours before operation since a high sulfanilamide concentration of the blood seemed desirable at the time of operation. Both drugs were discontinued for 16 hours after operation, following which they were again administered in approximately one-half of their former amounts. No accumulation of methemoglobin was detected throughout the period of observation.

This case demonstrates the prevention of methemoglobin accumulation in a patient receiving a large initial dose of sulfanilamide by administering a correspondingly large dose of methylene blue. Subsequent accumulation of methemoglobin was prevented by the administration of 2 grains of methylene blue every 4 hours during the period when she was receiving 0.2 gram of sulfanilamide per kgm. per 24 hours.

Case 5 Maurice McG., an 11 year-old white boy weighing 31.3 kgm., was admitted to the hospital with mastoiditis and lateral sinus phlebitis. He was given an initial dose of sulfanilamide of 0.2 gram per kilogram in 2 hours in 3 divided doses and thereafter received *0.2 gram per kgm. in 24 hours* in 6 divided doses. The methemoglobin was allowed to accumulate, and by the second day it had reached a value of 20 per cent of the total pigment, with a sulfanilamide concentration of the blood of 13.2 mgm per cent. At this time, he was given *intravenously* 1.5 *mgm.* of methylene blue per kgm The cyanosis rapidly decreased and was no longer detectable after 30 minutes. Beginning with the next dose of sulfanilamide, he was then given one grain of methylene blue every 4 hours. Twenty hours later the methemoglobin concentration was 10 per cent. Two hours after this observation and one-half hour before opera-

tion, the patient was again given methylene blue intravenously, the cyanosis which had recurred to a moderate extent again disappeared.

One dose of the drug was given after operation, then they were both discontinued for 12 hours, after which period the original dose of sulfanilamide was resumed, and the methylene blue was increased to 2 grains every 4 hours During the rest of the period of observation, which covered 6 days, the observed methemoglobin concentration ranged from an undetectable amount to 8 per cent, with the sulfanilamide concentrations of the blood ranging from 6 to 16 mgm per cent

This child represents a case in which the administration of one grain of methylene blue every 4 hours was insufficient to prevent the reaccumulation of methemoglobin following its reduction after the intravenous administration of the dye, but in which 2 grains every 4 hours retarded considerably the rate of reaccumulation.

Case 6 Hazel B, an 11-year-old white girl, weighing 40 kgm, was admitted to the hospital with a diagnosis of postscarlatinal cervical adenitis During the first 12 hours after admittance she received a total of 6 grams of sulfanilamide in 3 doses, and then was put on 0.2 gram per kgm per 24 hours in 6 divided doses Within 26 hours after the drug administration was started the methemoglobin concentration had reached a height of 27 per cent of the total pigment, the blood sulfanilamide being 13 9 mgm per cent At this time, she was given intravenously 1 5 mgm. of methylene blue per kilogram, and the methemoglobin concentration fell within 50 minutes to only 4 per cent of the total pigment From this time, the patient was given 2 grains of methylene blue every 4 hours, and the methemoglobin concentration on this day remained below 6 per cent. On the next day, doses of both drugs were cut to one-half of the original, and the methemoglobin concentration rose to 14 per cent of the total pigment. The dose of methylene blue was then increased to 2 grains every 4 hours, after which the methemoglobin concentration showed a gradual decrease, finally falling to a level below 9 per cent, where it remained for 3 days

This child showed an unusually high concentration of methemoglobin before the administration of methylene blue. The methemoglobinemia was almost completely eliminated by the intravenous administration of methylene blue, following which it was well controlled by 2 grains of methylene blue every 4 hours when the dose of sulfanilamide was 0 2 gram per kgm per 24 hours However, when the doses of both drugs were cut in half, the methemoglobinemia promptly increased and was again controlled only when the methylene blue dose was put back to its original level.

COMMENT

From an earlier study of some of the toxic effects of sulfanilamide (12), and from clinical observations, we do not feel that the relief or prevention of methemoglobinemia alleviates any of the other toxic symptoms The practical importance, therefore, of controlling methemoglobin accumulation and cyanosis rests upon certain other considerations It would seem definitely undesirable to deprive an extremely ill patient of any considerable amount of the oxygen carrying capacity of the blood We have shown that this deprivation may amount to as much as 37 per cent when moderately large doses of sulfanilamide are given, and such a reduction of oxygen carrying capacity would be extremely undesirable and perhaps dangerous in a patient with pulmonary disease, as pointed out by Bensley and Ross (13) Basman and Perley (10) have previously stated that when the oxygen capacity has dropped to a level equivalent to 8 grams of hemoglobin per 100 cc, they have considered it advisable either to discontinue the drug or reduce the dose, or to transfuse the patient The ready conversion of methemoglobin back to hemoglobin by the use of methylene blue makes resort to such measures unnecessary, and much larger doses of sulfanilamide may be tried without limitations imposed by this factor Even if oxygen want is not to be feared, the prevention of extreme cyanosis from the accumulation of methemoglobin is desirable in order to permit the recognition of possible cyanosis resulting from some other cause, and to make possible a better evaluation of the general clinical picture of the patient, which often appears deceptively alarming because of the methemoglobin cyanosis Ordinarily, the oral administration of methylene blue will satisfactorily control the methemoglobinemia, but if this should become appreciable, it may, under certain circumstances, be desirable to reduce the concentration rapidly This is particularly true in patients who must undergo anesthesia and operation Effective reduction of methemoglobin may be readily accomplished, as has been shown, by the intravenous administration of methylene blue.

As a result of the experiences just described, the following dosages of methylene blue may be recommended *In general, for children* weighing less than 20 kgm, 0 4 gram (6 grains) per day in 6 divided doses appears to be sufficient, at least for moderately large doses of sulfanilamide For children weighing over 20 kgm, a similar dosage is usually effective if as little as 0 1 gram per kgm per day of sulfanilamide is

being given In these children, if more sulfanilamide is being administered, 0 8 gram of methylene blue in 6 divided doses is recommended We feel that the accumulation of relatively small amounts of methemoglobin, up to 10 or 12 per cent of the total pigment, is of little consequence, certainly in patients who are not very ill Therefore, *the use of methylene blue in such patients* does not seem indicated unless a degree of cyanosis, suggesting considerable accumulation of methemoglobin should develop, especially in view of the fact that the discoloration of the lips by the drug itself and of the bed-clothing by the urine and vomitus is, to say the least, objectionable.

As mentioned before, the accumulation of methemoglobin is not entirely proportional to the dosage of sulfanilamide, so that these amounts of methylene blue may, in a given case, have to be altered, but in general these are the doses which we have found effective. When we are giving the sulfanilamide orally, we prefer to distribute its administration over the entire 24 hours, usually in 6 divided doses, and the methylene blue is given with each dose of sulfanilamide.

No serious intoxications from the use of methylene blue in man have been reported However, when the drug is given orally, even in moderate doses, it may cause vomiting and diarrhea, headache and tinnitus have also been reported (14) In spite of these effects, the drug may be considered only slightly toxic, and its continued use seems not to be harmful During intravenous administration of the drug, care must be taken to avoid perivenous infiltration, which is markedly painful and may lead to necrosis

Although no experimental data have been reported, from our clinical experience we feel that the simultaneous administration of methylene blue and sulfanilamide does not impair the therapeutic effectiveness of the latter

SUMMARY AND CONCLUSIONS

A study was made of factors leading both to the accumulation and disappearance of methemoglobin in patients to whom sulfanilamide was administered, and the following conclusions were reached

1 Reasonably close agreement exists between the direct spectroscopic determination of methemoglobin and the determination of non-oxygen carrying hemoglobin

2 In the great majority of patients receiving 0 1 gram or more of sulfanilamide per kgm per 24 hours, cyanosis develops, and so far we have been able to demonstrate the presence of methemoglobin in every case of cyanosis

3 There is marked individual variation in both the rate at which and the degree to which methemoglobin accumulates, although the dosage of sulfanilamide, its concentration in the blood, and perhaps also the extensiveness of the infection seem to have a direct relationship

4 Methylene blue causes a very rapid disappearance of cyanosis with simultaneous reduction in methemoglobin concentration, when given intravenously in single doses of 1 0 to 2.0 mgm per kgm, or when given orally in doses of 1 0 to 2 0 grains (65 to 130 mgm) repeated every 4 hours The latter method also prevents any appreciable formation of methemoglobin if started simultaneously with sulfanilamide administration

BIBLIOGRAPHY

1 Marshall, E. K. Jr., and Walzl E. M., On the cyanosis from sulfanilamide. Bull. Johns Hopkins Hosp. 1937 61, 140

2. Peters J P, and Van Slyke, D D., Quantitative Clinical Chemistry Vol. II. Methods. Williams and Wilkins Co., Baltimore, 1932 p 630

3 Archer H E., and Discombe, G, Sulphaemoglobinemia its cause and prevention, with special reference to treatment with sulphanilamide. Lancet, 1937, 2, 432.

4 Harrop, G A., and Waterfield, Reginald L., Sulphemoglobinemia. J A. M A., 1930 95 647

5 Paton J P J., and Eaton, J C., Sulphaemoglobinaemia and methaemoglobinaemia following administration of p-aminobenzenesulphonamide. Lancet, 1937, 1, 1159

6. Hauschild, F., Die Wirkung des Katalysins (Thionin) bei der Methamoglobinvergiftung Arch. f exper Path. u. Pharmakol., 1937, 184 458

7 Hauschild, F., Untersuchungen uber die Wirkung einiger Redoxyfarbstoffe bei Zustanden gestörter Atmung I Katalysin (Thionin) und Methamoglobinvergiftung Arch f exper Path. u. Pharmakol., 1936 182, 118.

8. Wendel, W B., Use of methylene blue in methemoglobinemia from sulfanilamide poisoning J A. M A (Correspondence) 1937, 109, 1216.

9 Peters J P., and Van Slyke, D D., Quantitative Clinical Chemistry Vol I Interpretations. Williams and Wilkins Co., Baltimore, [illegible]

10 Basman, J, and Perley, A. M, Report of patients treated with sulfanilamide at the St. Louis Children's Hospital J Pediat., 1937, 11, 212

11 Marshall, E K., Jr, Determination of sulfanilamide in blood and urine. Proc. Soc. Exper Biol and Med., 1937, 36, 422

12 Hartmann, A. F, Perley, A. M, and Barnett, H L, A study of some of the physiological effects of sulfanilamide. I Changes in the acid base balance. J Clin Invest., 1938, 17, 465

13 Bensley, E. H, and Ross, J B., Methemoglobinemia due to sulphanilamide therapy Canad. M A. J., 1937, 37, 62

14 Solis-Cohen, S, and Githens, T S, Pharmacotherapeutics, Materia Medica and Drug Addiction D Appleton and Co, New York, 1928, p 877

A PLETHYSMOGRAPHIC METHOD FOR THE QUANTITATIVE MEASUREMENT OF THE BLOOD FLOW IN THE FOOT[1]

BY EUGENE A. STEAD, JR. AND PAUL KUNKEL

(From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston)

(Received for publication June 10, 1938)

The importance of determining the blood flow in the foot in the study of vascular diseases involving the lower extremities is well recognized. Various methods, such as measurements of skin temperature, oscillometric tracings, the response to arterial occlusion, the histamine test, and tracings from toe plethysmographs, have been used to determine the functional capacity of the blood vessels of the foot. All these methods, however, yield only indirect indices of the total blood flow. The purpose of this investigation was to devise a method for the quantitative measurement of the blood flow in the foot in health and in disease. A plethysmograph has been designed which is based on the principle of Hewlett and Van Zwaluwenburg (1). The instrument is similar to that employed by Freeman (2) for measuring the blood flow in the hand. When the venous outflow is occluded by a "collecting" pressure lower than the diastolic pressure, the rate of initial increase in the foot volume is a measure of the amount of blood flowing to the foot.

Figure 1 shows the general design of the instrument. It is constructed of rigid brass sheets soldered together. The front presents an opening 15 cm. in diameter through which the foot is inserted. A wire grid supports the foot 2.5 cm. from the sides and the floor of the instrument. On the upper surface are openings for a thermometer and for a rubber tube which is connected with a Brodie bellows of 10 cc. capacity. Figure 2 is a sagittal section drawn to scale. The water is heated by two cartridge units of 100 and 200 watts capacity respectively. The heaters are enclosed in jackets tooled of solid brass. This precaution is necessary as soldered seams may leak and cause a short circuit in the heating unit. The jackets for the heaters are soldered into the plethysmograph beneath the grid and parallel to the back surface in such a position that they do not project beneath the heel where local heat may cause discomfort. A propeller for stirring the water is inserted through the back wall near its base. This is rotated by a shaft from a universal motor of 5000 r.p.m. with a gear ratio of 35 to 1. The electrical conductivity of the shaft is interrupted by a fiber joint. When two or more plethysmographs are employed they are connected by a copper wire soldered to the instruments and grounded.

The foot is inserted to the level of the malleoli through a thin rubber membrane having a cuff which fits lightly enough not to constrict the superficial veins and which is attached to the foot with rubber cement. The plethysmograph is placed at heart level. With the subject recumbent and the calf well supported in order to minimize pressure on the heel, the foot is inserted into the instrument and rests on a rubber pad. The rubber membrane is then stretched over the flanged opening of the instrument which has previously been coated with rubber cement and held in place by a metal ring making a watertight joint. A felt pad one half inch thick is adjusted to the ankle and is held rigid by an iris diaphragm of three brass plates which are secured by right angle clamps and wing nuts. The plethysmograph is filled with water at the desired temperature, and 60 cc. are then aspirated to allow for air transmission to the Brodie bellows. No determinations are made until the water bath has been at a constant temperature for 30 minutes. A pressure cuff 4 cm. in width is applied to the leg just proximal to the plethysmograph. This cuff may be inflated very rapidly from a 20-liter bottle fitted with a pressure gauge. In applying the rubber membrane,

[1] The expenses of this investigation were defrayed in part by a grant from the Proctor Fund of Harvard University for the study of chronic diseases.

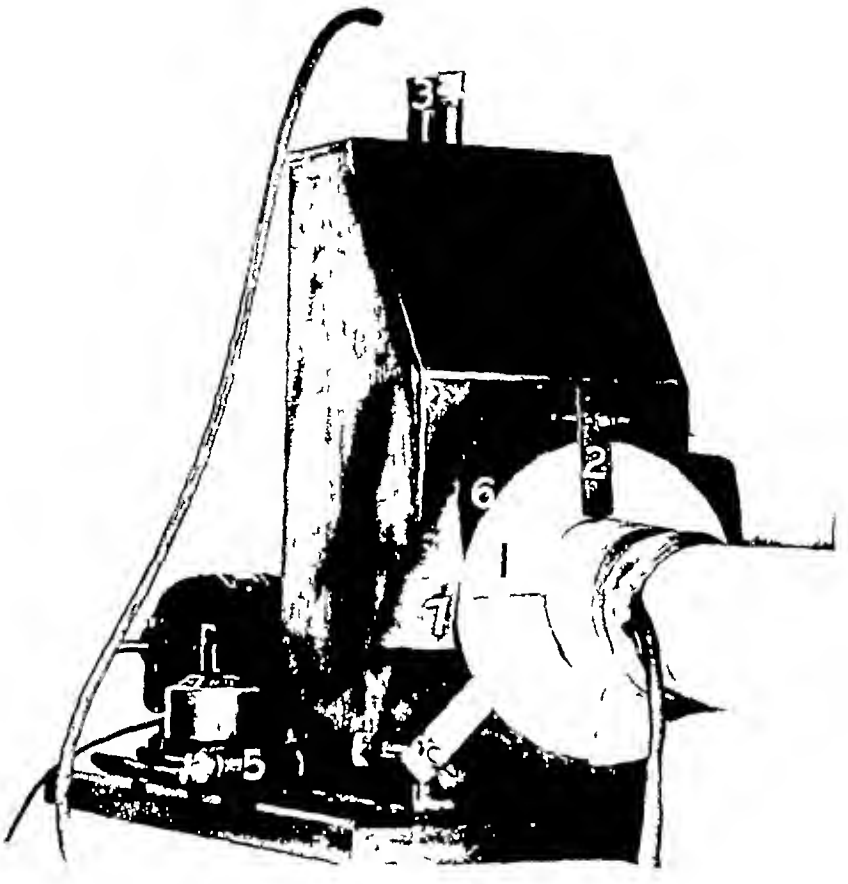


FIG 1 PLETHYSMOGRAPH WITH FOOT IN PLACE

(1) Iris diaphragm, (2) right-angle clamp and wing nut for securing iris diaphragm, (3) outlet for thermometer, (4) outlet for rubber tube to Brodie bellows, (5) cartridge unit heater (partially inserted), (6) felt pad, (7) rubber membrane, (8) pressure cuff

and the pressure cuff, care is taken not to cause an elevation of the venous pressure When sudden pressure is applied in the cuff a smooth, rising curve is traced on the smoked drum, the slope of which represents the rate at which the blood is flowing into the foot

In practice, the system is calibrated on a slowly revolving drum by adding 5 cc of air directly to the rubber tube which connects the instrument with the recording bellows The tube is clamped at the plethysmograph This method gives a greater deflection of the recording lever than is obtained when air is displaced by increasing the volume of water in the plethysmograph This introduces one source of error into the determinations, since during the recording of the blood flow, air is displaced into the recording system by the increase in the volume of the foot A second source of error results from the inertia of the plethysmograph-bellows system This is not apparent during the calibration on a slow drum, but it becomes obvious with the more rapid changes in volume associated with the actual determinations of blood flow In order to establish the correction factor for these two sources of error, six experiments were done with the foot in place The water in the plethysmograph was kept between 17 and 20° C in order to minimize the spontaneous changes in foot volume In these experiments water was added to the instrument at a known rate by a constant injection apparatus The tracings were recorded in the usual

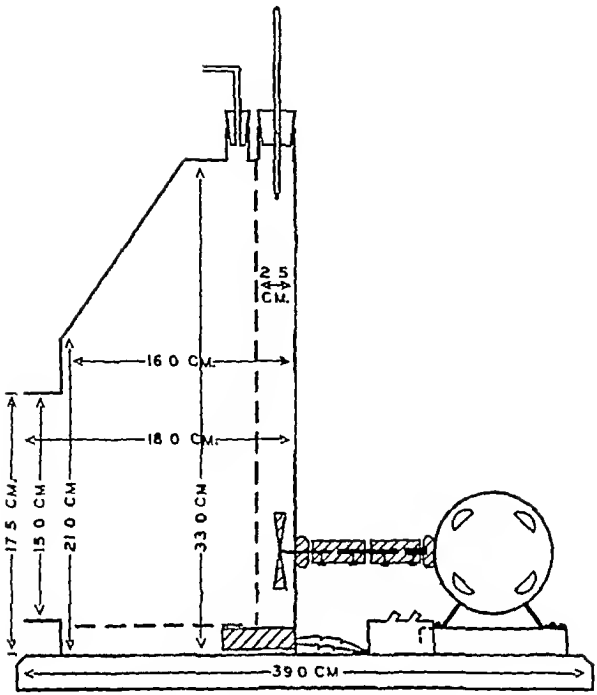


FIG. 2 SAGITTAL SECTION DRAWN TO SCALE

manner The amount of water added in unit time was calculated by the usual method of calibration and was compared with the amount of water actually added It was found that the actual increase in the volume of water was from 10 per cent to 16 per cent or an average of 13 per cent greater than the increase calculated from an average of five tracings in each experiment Therefore, in order to compensate for these two errors 13 per cent has been added to the flows as calculated from the air calibration With the foregoing correction these figures show an actual instrumental variation of ± 3 per cent when the flows are calculated as an average of five determinations The error of the method cannot be determined from the amount of variation between the individual blood flow curves since the variations resulting from changes in vasomotor tone are considerably greater than the error of the instrument

Figure 3 shows a blood flow tracing with the lines drawn for the calculation After projecting the slope of the curve to the base line, the number of seconds required for the curve to rise a distance equivalent to 3 cc is determined (this height is obtained by the introduction of 3 cc of air into the recording system) The volume of the foot in cubic centimeters is determined by subtracting from the volume of the instrument the amount of water necessary to fill the plethysmograph when the foot is in place To reduce the blood flow to cubic centimeters per minute per 100 cc of foot the following formula is used

$$\text{Blood flow} = \frac{H\,60}{S\,V} \times 1.13$$

H is the volume increase in cubic centimeters in S seconds V is the volume of foot in 100 cc or fraction thereof Sub tl es in Figure 3 we have

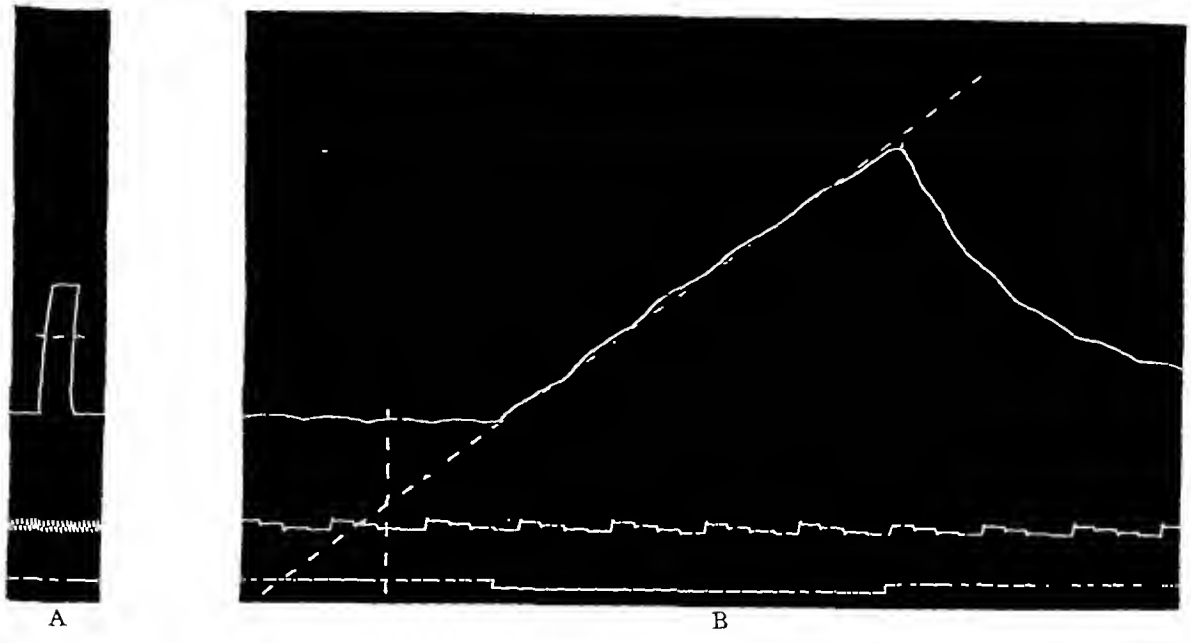


FIG 3

A Calibration on slowly revolving drum by adding 5 cc. of air to bellows Horizontal broken line at 3 cc mark *B* Typical blood flow curve with broken line continuing slope of curve to base line and interrupted at a distance equivalent to 3 cc obtained from calibration (Figure 3*A*) Drop in base line indicates period during which cuff pressure was applied

$$\text{Blood flow} = \frac{3 \times 60}{1\,12 \times 10\,10} \times 1\,13 = 18 \text{ cc per minute per 100 cc of foot}$$

An attempt was made to determine whether the 4 cm cuff at the ankle is adequate to block the venous return from the foot completely at the moment the pressure is applied When the 4 cm cuff was replaced either by one 12 cm cuff, or by two 12 cm cuffs applied about the midcalf and the lower thigh, respectively, no increase in flow was obtained over that observed with the 4 cm cuff

In normal persons, accurate curves are obtained with occluding pressures as high as the brachial diastolic pressure In the presence of obliterative vascular disease, however, care must be taken that the occluding pressure does not prevent the inflow of the arterial blood This may happen before the collecting pressure is raised to the brachial diastolic level In these cases the collecting pressure that gives the greatest constant increase in foot volume is used The ankle cuff is carefully adjusted so as not to displace the foot in the plethysmograph when the pressure is applied At times some displacement cannot be avoided, and in such cases the first part of the slope is disregarded

The instrument is sensitive enough to record pulse waves, respiratory waves, and changes in volume caused by variations in vasomotor tone Thus, by using a drum at slow speed the vasomotor reactions of the foot can be studied in detail

SUMMARY

A plethysmographic method has been described for the quantitative measurement of the blood flow in the foot With a standard correction for the inertia of the plethysmograph-bellows system, the instrumental error was found to be ± 3 per cent The plethysmograph is also useful in the study of the vasomotor reactions of the vessels of the foot

BIBLIOGRAPHY

1 Hewlett, A W, and Van Zwaluwenburg, J G, The rate of blood flow in the arm Heart, 1909–10, 1, 87

2 Freeman, N E, The effect of temperature on the rate of blood flow in the normal and in the sympathectomized hand Am J Physiol, 1935, 113, 384

BLOOD FLOW AND VASOMOTOR REACTIONS IN THE FOOT IN HEALTH, IN ARTERIOSCLEROSIS, AND IN THROMBO-ANGIITIS OBLITERANS

By PAUL KUNKEL and EUGENE A STEAD, JR.

(From the Thorndike Memorial Laboratory Second and Fourth Medical Services (Harvard) Boston City Hospital and the Department of Medicine, Harvard Medical School Boston)

(Received for publication June 10, 1938)

In a previous communication (1) a plethysmographic method has been described for the quantitative measurement of the blood flow in the foot. In the present report the results of such measurements in normal persons and in patients with vascular disease due to arteriosclerosis or to thromboangiitis obliterans are recorded. The vasomotor reactions of the foot were also studied in normal subjects. As the hand is much less frequently involved in circulatory disturbances of the extremities, a comparison was made of the blood flow and the vasomotor reactions in the hand and foot.

METHOD

The plethysmographic method described by us (1) was used for the study of the foot, and that described by Freeman (2) for the hand. No attempt was made to establish basal conditions. The subjects reclined in the horizontal position in a quiet room, with the extremities to be tested resting comfortably in the plethysmographs at heart level. The water bath was maintained at a constant temperature for 30 minutes before readings were taken. The determinations, each of which represents an average of five tracings taken in rapid succession, were made at approximately 5 minute intervals. The figures given in this report are usually averages of two or three such determinations made after the blood flow had reached a plateau. All the values for blood flow in both the hand and foot are recorded as cubic centimeters of blood per minute per 100 cc. of tissue. Various temperatures were used for the study of the vasomotor reactions, but for the purpose of comparing the flow in the foot with that in the hand and later for comparing the flow in the normal foot with that in the abnormal foot, the water in the plethysmograph was kept at 43° C., a temperature easily tolerated by most subjects. After 30 minutes at 43° C. spontaneous changes in the flow produced by vasoconstrictor impulses were at a minimum and the environmental temperature had very little effect on the immersed part. Throughout this report the blood flow to either the hand or the foot at 43° C. is designated as the "maximal" flow.

In 6 subjects the surface area of the hand or the foot within the plethysmograph was determined by making a light plaster mold of the part. This mold was cut while still soft and made to lie flat by multiple incisions. The outline of the mold was traced on cardboard, which was weighed. The surface area of the hand or the foot was calculated from the known weight of 100 sq. cm. of the cardboard.

Normal subjects

Maximal blood flow in the foot. A group of 34 normal subjects ranging in age from 17 to 67 years was selected from convalescent patients and house staff. The subjects had normal cardiovascular systems by the usual methods of clinical examination with the exception of 3 persons in the seventh decade of life who had some thickening of the radial arteries. The maximal blood flow in the 48 feet examined in this group averaged 17.1 cc., with the highest value 25.9 and the lowest 11.1 cc. The average maximal flow in the 33 feet from 23 normal males ranging in age from 17 to 67 years was 16.3 cc., with the highest 20.9 and the lowest 11.1 cc., and the average in 15 feet from 11 females between 17 and 50 years of age was 18.7 cc., with the highest 25.9 and the lowest 13.4 cc. Killian and Oclassen (3) used a plethysmograph for measuring the blood flow in the [illegible] could not be compar[illegible]

the flows were reported as the increase in the volume of the foot in cubic centimeters per minute The flow in the one subject at a comparable temperature (44 8° C), reported as cubic centimeters per minute per 100 cc of foot, was 14 8 cc

The variation in the maximal blood flow of the foot in different subjects was less than the variation in hand flows under similar conditions Blood flows in 90 per cent of the feet were between 13 and 20 cc., in only one instance was the flow less than 13 cc (Figure 1) There was no correlation between the blood flow of the foot and advancing age (Figure 2) The individual subjects showed considerable overlapping in all decades, and the differences in the average blood flow for the various decades were therefore not significant No persons over 70 years of age with normal cardiovascular systems were available for study Pickering (4), using Stewart's method of calorimetry, determined the rate of blood flow through the hand and concluded that it declined in subjects with normal blood pressure as age advanced He attributed this fall to sclerotic changes in the vessels of the hand This conclusion would undoubtedly hold true for the foot if normal blood pressure were the chief criterion used in the selection of normal subjects over 50 years of age We have not regarded as normal any person with an appreciable degree of arteriosclerosis, even though this places many symptomless subjects in the abnormal group

In 12 normal subjects the maximal blood flow was determined in both feet simultaneously The average difference was 1 8 cc and the greatest difference 4 5 cc In 2 subjects the maximal blood flow to the same foot was measured on three different days The values in one ranged

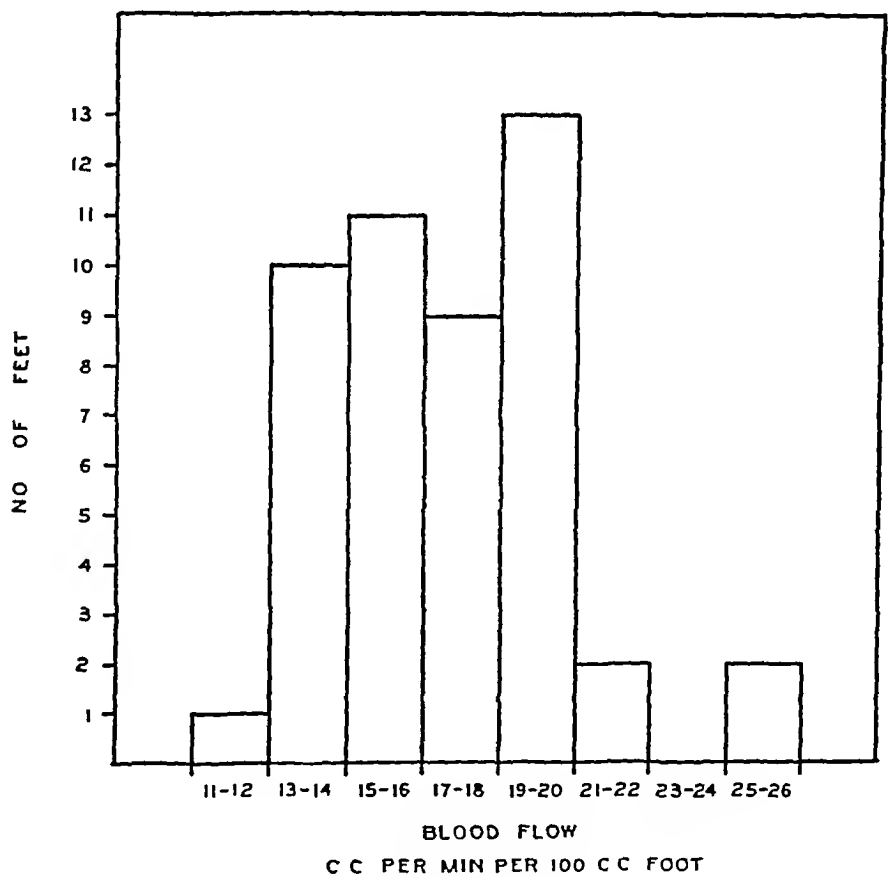


FIG 1 DISTRIBUTION OF 48 MAXIMAL BLOOD FLOWS IN THE FEET IN 34 NORMAL PERSONS

Cubic centimeters of blood per minute per 100 cc. of foot are plotted against the number of feet. Ninety per cent of the flows are between 13 and 20 cc.

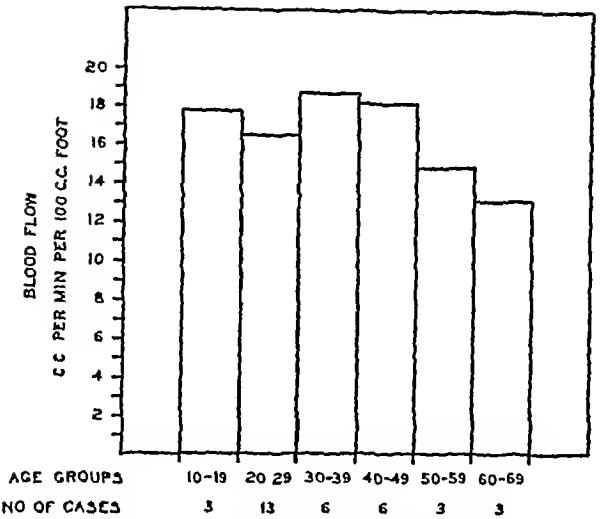


FIG. 2. RELATIONSHIP IN 34 NORMAL PERSONS BETWEEN AGE AND MAXIMAL BLOOD FLOW IN CUBIC CENTIMETERS PER MINUTE PER 100 CC. OF FOOT

from 13 4 to 15.7 cc with an average of 14 6 cc, and in the other from 13.2 to 15.2 cc. with an average of 14 4 cc. In 3 other instances determinations made on the same foot on two different days showed a maximum change in blood flow of 1 6 cc In 1 subject flows of 17 2 and 21 4 cc. were obtained in the right and the left foot, respectively The subject was wide awake and talkative, and had an average blood pressure of 123/80 and a pulse rate of 76 Two weeks later the flows were 13 1 and 15 7 cc., respectively, the flow in the right foot still being approximately 80 per cent of that in the left foot The patient was drowsy but not asleep, the average blood pressure was 110/80, and the pulse rate 62 The fall in flow on the second examination therefore appeared to be due to a change in cardiac output

Simultaneous blood flow measurements in the hand and foot Local circulatory disturbances are much more common in the foot than in the hand. This difference in incidence might be the result of (1) a special predisposition of the vessels of the foot to vascular diseases, (2) a congenitally greater blood supply to the hand, so that with equal degrees of circulatory impairment symptoms would develop first in the foot, or (3) a combination of these two factors Therefore, in 18 normal subjects maximal blood flows were determined in the hand and the foot at the same time. The average blood flow in the hand was 32 cc. with the highest value 54 4 and the lowest 18 7 cc., the average blood flow in the foot was 15 7 cc. with the highest value 19 5 and the lowest 11 1 cc. The fact that the maximal blood flow in the hand per unit of tissue was twice that in the foot seemed to indicate that at least one of the factors was the congenital difference in the blood supply

As the bones of the foot are larger than those of the hand, an attempt was made to obtain information from the literature on the relative volume of the bones of the foot and of the hand, but this was not successful As most of the blood flow in both the hand and foot is to the skin, an indirect approach to the problem was made by expressing the flows in relation to the skin area of the extremity instead of to the volume In 6 cases the surface areas of the hand and foot en
measured an

centimeters of blood per minute per 100 sq cm of skin This gave the average blood flow in the hand as 32 3 cc and the average blood flow in the foot as 24 8 cc The blood flow to the hand calculated in this manner was 30 per cent greater than the blood flow to the foot The subjects usually stated that at the same temperature (43° C) the foot felt warmer than the hand It is possible that this was owing to the smaller blood flow in the foot and the less rapid cooling of the tissues by the blood stream

Vasomotor reactions of the foot and the hand The blood flow in the foot was greatly modified (*a*) by the temperature of the surrounding water and (*b*) by the changes in the environmental temperature of the body with the foot at local temperatures which did not cause extreme vasodilatation or constriction At temperatures as low as from 17° to 20° C the flow dropped to about 0 2 cc per minute per 100 cc of foot The spontaneous vasomotor variations practically disappeared, but the respiratory waves, not being produced by changes in vasomotor tone, persisted With the bath at this low temperature the vessels constricted so tightly that the flow was not detectably modified by changing the temperature of the environment of the subject or by immersing the hands in water at 45° C The blood flow dropped to such low levels that the small amount of chilled blood returned from the cold foot had no detectable effect on the heat regulating mechanism, and generalized sweating was easily induced by heating the body

At temperatures of the water bath from 32° to 37° C the blood flow to the foot was greatly influenced by the amount of generalized cutaneous vasodilatation present In subjects without generalized vasodilatation, in a cool room the blood flow averaged about 1 cc at 32° C and about 5 cc at 37° C When generalized cutaneous vasodilatation was induced by blankets and hot water bottles or by immersing the hands in hot water, the blood flow rose to a level of from 8 to 12 cc The blood flow in the foot at 37° C caused by heating the body was rarely greater than one-half that produced by a local heat of 43° C There was, however, considerable individual variation and in 1 subject heating the body produced such marked cutaneous vasodilatation that the blood flow at 37° C nearly equaled that at 43° C In both the hands and the feet, at 32° C, the blood flows were about 1 cc. in the absence of generalized vasodilatation When the body was heated, the blood flow in the hand usually increased much sooner than in the foot This is in accord with the common observation of warm hands and cold feet

The vessels of the feet responded by vasoconstriction to psychic influences and pinching the skin in essentially the same manner as those of the hand No effort was made to quantitate the stimuli, or to determine whether or not reflex vasoconstriction could be limited to either the foot or the hand by the use of appropriate stimuli applied to various points on the body At 43° C the vessels of the foot and of the hands of different subjects showed marked variation in the response to the pinch stimulus Some subjects lost the pinch response completely, while in a few it was as active as at 37° C

In order to determine whether the local heat of 43° C caused maximal dilatation of the vessels of the foot, the heat stimulus was reinforced by a 5-minute period of arterial occlusion Wide blood pressure cuffs were applied just above the collecting cuff and just below the knee These were suddenly inflated from a large reservoir to a pressure of 290 mm Hg In each instance the arterial occlusion was complete, as was shown by the absence of any increase in foot volume Measurements of blood flow were made immediately following release of the pressure, at a time when the vascular dilatation caused by the reactive hyperemia should have been at its height. In 2 subjects there was no increase in blood flow, in a third it increased from 22 9 to 26 4 cc Thus the blood flows obtained with the foot bath at 43° C are nearly maximal

The tracings of the blood flow from the foot at 43° C showed a constant increase in volume for a longer time than those from the hand at the same temperature and were therefore easier to interpret This difference in behavior of the hand and foot curves was not owing to any difference in the reaction of the vessels to heat, but rather to technical differences In the hand, at temperatures from 43° to 45° C, as noted by Capps (5) the amount of venous distention ob-

tained by a given degree of venous obstruction was frequently less than at lower temperatures (37° C.) The veins even though dilated remained full as the result of the rapid inflow of arterial blood. The amount of available venous space for the increase in the hand volume when the collecting pressure was applied was small and the tracings were straight for only a short distance. This difficulty has not been encountered in the foot. The slower blood flow prevented the veins from becoming engorged to as great a degree as in the hand and the greater hydrostatic pressure resulting from the higher column of water in the foot plethysmograph caused the veins of the foot to empty more rapidly. That these factors were operative is indicated by experiments with the hand at 43° in which the apparent decrease in the amount of venous distention for a given degree of venous obstruction was increased over that present at 37° by raising the hydrostatic pressure in the hand plethysmograph to the same level as that used in the foot plethysmograph. The curves of the blood flow in the hand likewise became straighter for a longer distance.

The identical response of the vasomotor system in the hand and foot was also demonstrated by the simultaneous drop in the base line of the tracings of the hand and foot when vasoconstriction was induced by a deep inspiration (Figure 3*A*). Bolton, Carmichael and Stürup (6) have called attention to this reflex in the toes and the fingers. On the other hand, the usual respiratory waves that were frequently visible on a slow drum even at low temperatures (17° C.) were not of vasomotor origin. Figure 3*B* shows these waves in a subject breathing more deeply than normally. The elevations in the hand waves occurred at the same time as the depressions in the tracings of the foot. During inspiration the negative pres

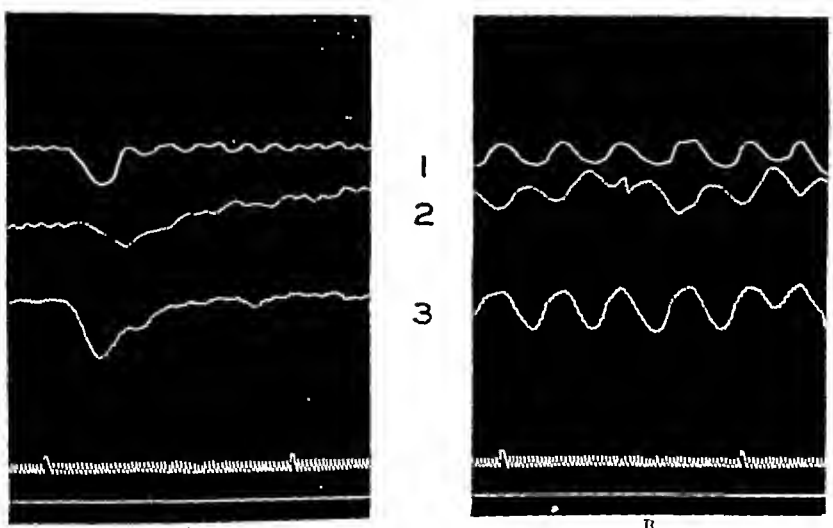


FIG. 3*A*. VASOCONSTRICTION IN BOTH HAND AND FOOT FOLLOWING DEEP INSPIRATION. (1) RESPIRATORY TRACING WITH DOWN STROKE INDICATING INSPIRATION. (2) TRACING FROM FOOT. (3) TRACING FROM HAND. IN (2) AND (3) A DOWNWARD DEFLECTION IS CAUSED BY A DECREASE IN THE VOLUME OF THE PART.

FIG. 3*B*. RESPIRATORY WAVES FROM HAND AND FOOT TRACINGS EXAGGERATED BY MODERATELY DEEP BREATHING.

During inspiration the foot increases and the hand decreases in volume; during expiration the foot decreases and the hand increases in volume.

sure in the thorax was increased, the venous pressure in the arm fell with a resulting decrease in the volume of the hand At the same time the intra-abdominal pressure rose, the venous pressure in the leg increased, and the foot increased in volume During expiration, the volume of the hand increased and the volume of the foot decreased These shifts again followed the changes in intrathoracic and intra-abdominal pressures Lluesma Uranga (7) called attention to the fact that these waves were reversed in the upper and lower extremities but offered no explanation for the phenomenon

Patients with arteriosclerosis and thromboangiitis obliterans

Twenty-one feet with arteriosclerosis of the vessels of the foot were studied in 13 subjects A summary of the relevant clinical data and of the blood flows is given in Table I No pulsations of the posterior tibial arteries were palpable in these subjects In 1 of the 3 cases in whom the dorsalis pedis pulsations were equal bilaterally the vessel walls were palpable and sclerosed, in the other 2 there was evidence of sclerosis of the radial, brachial, and cerebral vessels The 10 feet without symptoms and without trophic disturbances had an average maximal flow of 8 cc In the 11 feet with either symptoms or trophic disturbances the flow averaged 4 8 cc

Thus, in general, the maximal flow to the foot can be decreased 50 per cent without producing any signs or symptoms When the maximal flow is decreased to approximately one-third the normal value, or to the level of 5 cc or below, symptoms and trophic changes are apt to occur

TABLE I

Clinical data and maximal blood flow in the feet in 13 patients with arteriosclerosis of the vessels of the foot

Case number	Age	Symptoms		Trophic disturbances in the skin		Arterial pulsations				Maximal blood flow (cc per minute per 100 cc of foot)		Remarks
						Right		Left				
		Right	Left	Right	Left	Dorsalis pedis	Posterior tibial	Dorsalis pedis	Posterior tibial	Right	Left	
1	*years* 70	None	None	None	None	Present	Absent	Present	Absent	6 7	6.5	Activities restricted to home
2	77	None	None	None	None	Present	Absent	Present	Absent	8.2	Not determined	Activities restricted to home
3	79	None	None	None	None	Present	Absent	Present	Absent	Not determined	6 7	Activities restricted to home
4	71	None	None	Dry and atrophic	Dry and atrophic	Present	Absent	Absent	Absent	5 8	3.3	Activities restricted to home
5	78	None	None	None	None	Absent	Absent	Absent	Absent	8 8	9 0	Active
6	57	Mid-thigh amputation 2 years ago	None		Red, thin and atrophic			Absent	Absent		5 3	Ambulatory but not very active
7	69	Mid-thigh amputation 4 years ago	None		Dry atrophic with numerous small dilated veins			Absent	Absent		4 1	Diabetic Activity practically restricted to home
8	46	Foot sensitive to cold	Mid-thigh amputation 6 months ago	Pale and cold		Absent	Absent			4 7		Diabetic. Ambulatory
9	67	One toe amputated 1 year ago	Toes painful	None	Middle toe discolored. Dorsum of foot red	Present	Absent	Very faint	Absent	8 0	5 5	Diabetic. Ambulatory
10	60	None	Foot cold for 6 months	None	None	Present	Absent	Absent	Absent	9.8	6.8	Ambulatory and *fairly active*
11	64	Foot very painful for 2 months. Relieved by heat	None	Persistently cold and very pale	None	Absent	Absent	Absent	Absent	4.0	9 7	Ambulatory and active
12	69	Continual pain in feet		Cold, shiny and atrophic	Shiny and atrophic. Middle 3 toes very cold and dusky	Absent	Absent	Absent	Absent	3 0	2 0	Bedridden
13	58	None in feet. Intermittent claudication of calf		None	None	Absent	Absent	Absent	Absent	7 9	6 7	Activity markedly limited by pain in calves

The exact level at which these appear is influenced to a large extent by the general activity of the subject and by the exposure to cold and trauma to which the foot is subjected Thus no symptoms were present in Cases 1 through 4 in whom there was an average blood flow of 6.2 cc. The activities of this group of subjects were restricted to their homes through weakness and senility In the other 9 feet with blood flows under 7 cc. only 3 were symptomless and in each patient the range of activities was greatly limited, in 2 by mid-thigh amputations and in the third by intermittent claudication in both calves

The patient in Table I with intermittent claudication in both calves had reduced blood flow in both feet Studies were also made on a 55 year old man with a typical history of intermittent claudication in the right calf of 3 years' duration but with no symptoms in the left calf He complained also of cold, purple feet on exposure to low temperatures The feet appeared normal at room temperature (22° C) Both dorsalis pedis and posterior tibial pulsations were present though they were somewhat less forceful on the right The maximal blood flow in the right foot was 14.9 cc. and that in the left 26.3 cc. The change in color and the pain at low temperature were probably caused by vascular spasm It was assumed that the blood flow to the calf muscles on the right had been curtailed to a much greater extent than the flow to the right foot, which was still well within normal limits though decreased for this particular individual, as was shown by the unusually rapid blood flow in the left foot. Thus if the arteriosclerotic changes occur in localized areas, or if adequate collateral circulation is established, severe intermittent claudication may occur in the calf while the blood flow in the foot is still as rapid as in many normal individuals

Table II gives the clinical data and blood flow in the feet of 5 cases of thromboangiitis obliterans The diagnosis seemed to be definitely established in the first 4 cases The etiology of the vascular disease in the fifth case was in some doubt but, in the absence of any peripheral sclerosis demonstrable either by palpation or by x-ray examination of the lower extremities, it has been included in the group with thromboangiitis obliterans When the vessels of the feet are involved in this disease the symptoms and trophic disturbances are produced at about the same level of blood flow as in the arteriosclerotic group Thus the 2 feet with blood flows below 6 cc showed either trophic disturbances or sensitivity to cold As in the arteriosclerotic group, intermittent claudication of the calf may be incapacitating while the collateral circulation is sufficient to keep

TABLE II

Clinical data and maximal blood flow in the feet in 5 patients with thromboangiitis obliterans

Case number	Age	Duration and severity of intermittent claudication in calves		Distance before pain halts patient	Symptoms and signs of trophic disturbances in foot		Arterial pulsations				Maximal blood flow (cc. per minute per 100 cc of foot)	
							Right		Left			
		Right	Left		Right	Left	Dorsalis pedis	Posterior tibial	Dorsalis pedis	Posterior tibial	Right	Left
	years			yards								
1	31	5 years severe	5 years severe	200	None	Skin discolored and pigmented	Absent	Absent	Absent	Absent	10 1	4 9
2	32	10 years severe	10 years severe	200	Sensitive to cold Dusky red	Very sensitive to cold Ulcer on one toe	Absent	Absent	Absent	Absent	5 8	Not determined
3	48	9 years severe	No difficulty	1000	None	None	Absent	Absent	Present	Present	12 8	24.9
4	49	25 years severe	25 years severe	100	None	Ulcer on toe and ankle	Absent	Absent	Absent	Absent	6.8	Not determined
5	57	Questionable	2 years severe	200	None	None	Absent	Very faint	Absent	Absent	11.0	6.2

the blood flow in the foot within average normal limits When only one leg is involved, however, this flow can be shown to be low for that particular individual by comparing it with the normal foot In the cases of arteriosclerosis and thromboangiitis obliterans no determinations were made on feet with open ulcers As a rule the patients tolerated the local heat quite well, although in 4 cases the water was allowed to cool down to 42° C because the heat caused local discomfort In no case did the skin show any ill effects from the prolonged soaking in hot water

DISCUSSION

The plethysmographic method of studying the circulation in the foot has an advantage over the indirect methods, such as skin temperature measurements, reactive hyperemia, histamine test, and toe plethysmograph tracings, for blood flow is measured directly and recorded as cubic centimeters of blood per minute per 100 cc of tissue Therefore, direct comparison can be made not only between blood flow in the normal and the abnormal foot, but also between the blood flow in the foot and in other parts of the body The method offers a quantitative means for following the natural course of vascular disease in the foot and for studying the efficacy of various forms of therapy The production of the maximal blood flow in the foot by local heat obviates the necessity for careful regulation of the room temperature As the direct heat overcomes the vascular spasm, spinal anesthesia or nerve block is not necessary for the evaluation of the degree of vascular change in those cases in whom vasodilatation is not produced by heating the body The plethysmographic method has the disadvantage, under certain conditions, of recording the blood flow in the entire foot Thus, with the pathology localized in one toe, the readings would be within normal limits It may be asked whether the circulation is measured in those cases in whom the blood is brought to the foot through small vessels in which the pressure may drop to much lower levels than in the larger vessels ordinarily supplying the foot with blood The occurrence of blood flows of from 9 to 13 cc. in cases without palpable pulsation in the vessels of the foot, and the correlation of signs and symptoms with similar levels of blood flow in cases with arteriosclerosis and thromboangiitis obliterans, indicate that the collateral flow is measured when present In these measurements of total blood flow in the foot no distinction is made between blood flowing through capillaries and that flowing through arteriovenous anastomoses Thus the total blood flow may not always indicate accurately the amount of blood available for nourishing the tissues

SUMMARY AND CONCLUSIONS

1 Measurements of the blood flow in the foot in health, in arteriosclerosis, and in thromboangiitis obliterans were made under standard conditions by the plethysmographic method The flow was recorded as cubic centimeters of blood per minute per 100 cc of tissue

2 The blood flow to the foot reached a constant level after 30 minutes at 43° C The flow at this temperature has been designated as the "*maximal*" *flow*

3 The average maximal blood flow to the foot in normal persons was 17 1 cc, with the highest 25 9 and the lowest 11 1 cc Ninety per cent of the flows were between 13 and 20 cc The average difference in the maximal flow in the right and left foot was 1 8 cc

4 The maximal blood flow in the foot showed no appreciable decrease with age (17 to 67 years) in the presence of a normal cardiovascular system

5 The average maximal blood flow in the hand per equal volume of tissue was twice that in the foot When calculated in relation to skin area the maximal flow in the hand was 30 per cent greater than that in the foot

6 The vasomotor reactions of the hand and foot were qualitatively similar The rhythmic respiratory waves observed during normal breathing resulted from the changes in venous pressure associated with respiration and were not of vasomotor origin A deep inspiration, however, induced constriction of vasomotor origin in both the hand and the foot

7 In arteriosclerosis and thromboangiitis obliterans the maximal blood flow to the foot was reduced 50 per cent without symptoms or trophic disturbances When the flow was reduced to one-third the normal value, or to the level of 5 cc.

or below, symptoms or trophic disturbances usually occurred

8. In both arteriosclerosis and thromboangiitis obliterans severe intermittent claudication in the calf was in some cases incapacitating, though the blood flow in the foot was as great as in many normal individuals Thus the presence of an adequate supply of blood to the foot did not eliminate the possibility of obliterative disease involving the vessels of the calf muscles

The authors wish to express their sincere appreciation to Dr Soma Weiss for his many helpful suggestions and guidance in this work, and to Miss Sophia M Simmons, for her technical aid. They wish also to thank Dr Edward A. Edwards for sending them two cases of thromboangiitis obliterans.

BIBLIOGRAPHY

1 Stead, E. A., Jr and Kunkel, P A plethysmographic method for the quantitative measurement of the blood flow in the foot. J Clin. Invest., 1938, 17, 711

2. Freeman, N E., The effect of temperature on the rate of blood flow in the normal and in the sympathectomized hand. Am. J Physiol., 1935 113, 384

3 Killian, J A., and Oclassen, C. A., Comparative effects of water baths and mustard baths at varying temperatures on the rate of peripheral blood flow in man. Am. Heart J., 1938 15, 425

4 Pickering G W The peripheral resistance in persistent arterial hypertension. Clin. Sc., 1936 2, 209

5 Capps R. B., A method for measuring tone and reflex constriction of the capillaries venules and veins of the human hand with the results in normal and diseased states *J Clin. Invest., 1936 15, 229*

6. Bolton, B., Carmichael E. A., and Sturup G, Vasoconstriction following deep inspiration. J Physiol., 1936 86, 83

7 Lluesma Uranga, E. El método pletismográfico en la determinación de la capacidad funcional de las obstrucciones y espasmos arteriales de los miembros Crón. méd., Valencia, 1934 38, 415

THE INSULIN AND THE ZINC CONTENT OF NORMAL AND DIABETIC PANCREAS

By D A. SCOTT and A. M FISHER

(From the Connaught Laboratories University of Toronto, Toronto, Canada)

(Received for publication June 13, 1938)

Although insulin, the antidiabetic principle of the pancreas, was discovered in 1921 (1) there is no record, so far as we are aware, of any study comparing the insulin content of the pancreas of normal individuals with that of the pancreas of persons suffering from diabetes mellitus The reason for the lack of information on this phase of the study of diabetes is probably owing to an assumption that, since the condition of the diabetic is suggestive of a shortage of insulin, the pancreas *must* contain less insulin than is normally present However, other explanations for an apparent shortage of insulin would also seem feasible For example, it might be suggested that, in the diabetic patient, the insulin is stored in, or excreted from, the pancreas in a form which the body is not able to utilize. Again, the apparent deficiency of insulin may be due, not to any abnormality in the excretion of the hormone itself, but rather to the liberation of an excessive amount of one or more substances which modify the action of the hormone It is known that the blood-sugar-lowering action of insulin can be greatly modified by the addition, *in vitro* of various constituents of pancreas, blood, and thymus gland Zinc, spermine (both normally present in pancreas), a preparation from blood and another from thymus gland have all been shown to prolong or even annul this action of the hormone (2, 3, 4, 5, 6) On the other hand, it could be suggested that diabetes is concerned not with an abnormal functioning of the pancreas but with a disorder in some other gland such as a pituitary disturbance (7 8) In view of the foregoing, it was a matter of interest to ascertain the insulin content of the pancreas of a series of normal and diabetic persons Because of the close association between insulin and zinc, as shown in publications from these Laboratories (9, 10), it was decided to estimate the zinc content of each pancreas Since the liver also is concerned with carbohydrate metabolism, the zinc content of this organ was likewise determined.

EXPERIMENTAL

Over a period of several months, a series of 14 pancreases was obtained at the autopsy of individuals who had met almost instantaneous death resulting from accidents or other causes. These *subjects had no history* of diabetes and may be taken to represent as nearly as possible a normal group of individuals At each autopsy a sample of liver was also obtained.

Another series of 18 pancreases was obtained at autopsy of individuals who had a history of diabetes mellitus usually of some years' duration. All the patients were receiving insulin daily The severity of the disease ranged from mild to moderately severe. Diabetes was a contributory rather than a primary cause of death. The time elapsing between death and autopsy was as in the control group, approximately 10 hours. A sample of liver was also obtained at each autopsy

Immediately on obtaining the pancreas and liver they were taken to the laboratory and the pancreas weighed The zinc and the insulin content of each pancreas and the zinc content of the liver was estimated as described below

Zinc estimations All samples of pancreas and liver for zinc estimations were digested with 5 N HCl A sufficient quantity of this acid was prepared, for all the analyses recorded herein by distilling 20 per cent hydrochloric acid (C. P) in pyrex glassware, and diluting the distillate to the desired strength with distilled water A spectroscopic analysis of this solution gave a negative test for zinc.

The pancreas was cut into small pieces by means of stainless steel scissors. From this a 10 gram sample was weighed and transferred to a 100 cc. pyrex digestion flask. To this was added 50 cc. of the acid digestion liquid. This mixture was refluxed for 1 hour and was then allowed to stand overnight. The following morning the volume was measured and the mixture filtered through acid washed filter paper The zinc content of an aliquot of the filtrate was estimated spectroscopically and the amount of zinc per gram of tissue calculated. The zinc content per gram of liver was calculated from a similar digestion carried out on 10 grams of liver with *50 cc. of the acid digestion mixture.* The results of these estimations are shown in Tables 1 and II

The spectroscopic analyses were conducted by Dr S Bateson of the Department of Physics of this Uni-

versity Details of the method, limit of error ± 3 per cent, will be published elsewhere.

Insulin estimations Immediately after removal of the 10-gram sample of pancreas for the zinc estimation, the remainder was minced The material was then weighed into an Erlenmeyer flask and to it was added acid alcohol in the proportion of 2 5 cc. for each gram of pancreas (Several liters of the alcoholic extraction fluid were prepared and portions of it used for extracting each pancreas It consisted of 750 cc. of absolute alcohol, 15 cc. of concentrated hydrochloric acid, and 235 cc of water) After standing overnight the mixture was filtered through a double layer of cheese-cloth and pressed until nearly dry The solid material was again extracted for two hours with a volume of acid alcohol equal to that used in the first extraction The alcoholic extract was again filtered through cheese-cloth and the two extracts combined The filtrate was made slightly ammoniacal and the volume measured The mixture was filtered through filter paper and four 10 cc. quantities pipetted into each of four 50 cc. centrifuge thimbles To each tube were now added 15 cc of absolute alcohol and 25 cc. of ether The mixtures were shaken and placed in the refrigerator overnight. They were then centrifuged, the supernatant ether-alcohol discarded, and the tubes allowed to drain for one-half hour The precipitate in each tube was then dissolved in 10 cc. of isotonic saline (pH 2.5) The insulin in these solutions or of further dilutions of them was estimated by the mouse method of assay (11) Generally, six tests were conducted involving at least 300 mice. From the average of these potency values the number of units of insulin per gram of pancreas was calculated The limit of error in the assays was probably not greater than 10 per cent The insulin content of each pancreas is recorded in Tables I and II

TABLE I

The insulin and the zinc content of the pancreas of non-diabetics

Patient number	Sex	Age	Cause of death	Time elapsing between death and autopsy	Weight of pancreas	Insulin per gram of pancreas	Total insulin per pancreas	Zinc per gram of pancreas	Total zinc per pancreas	Zinc per gram of liver
		years		*hours*	*grams*	*units*	*units*	*mgm*	*mgm.*	*mgm*
1	M.	12	Auto accident	10	44	2 1	92	0.18	7 9	0.25
2	M.	14	Auto accident	9	116	1 9	220	0.13	15 1	0.22
3	M.	15	Drowned	12	40	2.5	100	0 13	5.2	0.25
4	F	19	Auto accident	10	68	1.8	122	0 13	8.8	0.25
5	M.	38	Auto accident	10	97	1 4	136	0.12	11 6	0.16
6	F	35	Street car accident	12	120	1.0	120	0 09	10 8	0.26
7	M	38	Fractured skull	12	74	8.8	279	0 15	11 1	0.19
8	M.	47	Alcoholic poisoning	10	248	0.6	149	0.20	59 6	0.18
9	M	52	Alcoholic poisoning	5	150	1.4	210	0.18	27 0	0.25
10	M.	55	Suicide	4	115	2.6	299	0 14	16.1	0.20
11	M	56	Fractured skull	8	115	2 0	230	0.24	27 6	0.26
12	M.	60	Auto accident	11	125	1 1	137	0.10	13.8	0.24
13	M.	72	Struck by train	12	263	0.8	210	0.11	28.9	0 15
14	M.	75	Coronary thrombosis	10	101	1.2	121	0 10	10.1	0 16
Average		41.2		9 6	119 7	1 7	173	0.14	18.1	0.22

TABLE II

The insulin and the zinc content of the pancreas of diabetics

Patient number	Sex	Age	Complications other than diabetes	Duration of diabetes	Weight of pancreas	Insulin per gram of pancreas	Total insulin per pancreas	Zinc per gram of pancreas	Total zinc per pancreas	Zinc per gram of liver	Remarks
		years		*years*	*grams*	*units*	*units*	*mgm*	*mgm*	*mgm*	
15	F	12		7	41	<0 08	< 3	0 07	2 9	0 19	Severe diabetic
16	F	19	Anuria	1	94	0 1	10	0 09	8 5	0 18	
17	M	28	Pneumonia	10	70	<0 03	< 3	0 09	6 3	0 17	Mild diabetic, fatty pancreas
18	M	44	Tuberculosis	5	64	0 3	19	0 09	5 8		Moderately severe diabetic
19	M	46	Septicemia	10	130			0 06	7 8		Pancreas preserved in formalin
20	M	55	Hyperthyroidism	1	86	0 3	26	0 10	8 6		Mild diabetic
21	F	56	Pneumonia	5	65	0 7	45	0 06	3 9	0 15	Mild diabetic
22	M	56	Hypertension	7	75	0 5	38	0 05	3 7	0 09	Mild diabetic
23	M	61	Coronary thrombosis	16	149	0 5	75	0 05	7 5	0 14	Mild diabetic, fatty pancreas
24	F	62	Adrenal tumor		85	0 4	34	0 08	6 8	0 16	
25	M	63	Septicemia	8	120	0 8	96	0 04	4 8	0 17	Mild diabetic
26	F	63	Carcinoma	3	190	0 1	19	0 05	9 5	0 20	Fatty pancreas
27	F	63	Carcinoma	4	70			0 10	7 0		Pancreas preserved in formalin
28	F	65	Arteriosclerosis	12	95	0 3	28	0 07	6 7	0 22	Mild diabetic
29	F	71	Arteriosclerosis	12	80	0 5	40	0 08	6 4	0 14	Mild diabetic
30	M	72	Auricular fibrillation	8	120	0 2	26	0 04	4 8	0 29	Moderately severe diabetic
31	F	74	Chronic myocarditis		110	0 2	26	0 11	12 1	0 19	
32	F	76	Arteriosclerosis	8	85	1 9	162	0 04	3 4		Moderately severe diabetic
Average		55		7	96	<0 4	<40	0 07	6 5	0 18	

DISCUSSION

It will be seen from Table I that the average value obtained for the insulin content of the pancreas of normal individuals is 1 7 international units per gram This value is in good agreement with that obtained in previous work (12) in which it was shown that the insulin content of the pancreas of mature cows is about 1 8 international units per gram Similarly, it has recently been found that, in cats, the insulin content of the pancreas is approximately 1.7 international units per gram It would appear that the insulin content of the pancreas of normal humans is unaffected by age, provided the individuals are at least 12 years old Such a result was not anticipated in view of a previous paper (12) showing that the insulin content of the pancreas of young calves is much greater than that of two-year old cattle However it may be that an investigation of the insulin content of the pancreas during infancy or early childhood would show that such pancreases contain much more insulin than was found in the series reported in the present paper It will be noted that two pancreases in this group (Patients 8 and 13) have less than one unit of insulin per gram of tissue These pancreases were fatty and were much heavier than the average normal pancreas Their total insulin content compares favorably with that found in the remaining pancreases in this group The zinc content per gram of tissue was found to be less in the pancreas than in the liver In normal cats, on the other hand, it has been found that the zinc content per gram of tissue is less in the liver than in the pancreas (13) However, in both cats and humans the total zinc content of the liver is many times that of the pancreas

In Table II it is shown that the average value for the insulin content of the pancreas of diabetics is less than 0 4 unit of insulin per gram of tissue Thus these glands contain less than one quarter the amount of insulin found in those of the normal group It will also be noted from Table II that there is a great variation in the concentration of insulin in the various pancreases Most of the pancreases contain between 0 1 and 0 5 unit of insulin per gram of tissue. There are two (Patients 15 and 17) having less than 0 1, and three (Patients 21, 24, and 32) with more than 0 5 unit of insulin per gram of pancreas It is surprising that the very low values occurred in the younger age group since we have shown that, in cattle, the lower values occur in the older age groups Patient 15 was admitted to the hospital in diabetic coma During the 36-hour interval before death she received 200 units of insulin with no clinical response Patient 17, although only a mild diabetic, had been extremely difficult to keep under proper control During the 24 hours prior to death this patient received 104 units of insulin From the insulin determinations it is evident that the injected insulin was not stored in the pancreas in either case Of the three patients having an insulin content of more than 0 5 unit of insulin per cc., Patient 32 is the most interesting and surprising This patient had been a moderately severe diabetic for many years but was kept under fairly good control with insulin Death was caused by extreme arterio sclerosis showing fibrosis of the myocardium and acute aortitis The pancreas was fibrous and showed marked arteriosclerosis Whether any of these complications is responsible for the extremely high insulin content of this pancreas is, in our opinion, doubtful At the present time we are quite unable to give any valid reasons for this diabetic having a pancreas with a normal insulin content The average zinc content of the diabetic pancreases is one-half the value obtained for the normal pancreases The concentration of zinc in the liver is only slightly less in the diabetic group than in the control group This is probably insignificant since the liver of many diabetics is enlarged and often contains a relatively large amount of fat. Hence the total zinc content of such livers might equal, or even exceed, that of the livers of normal individuals

SUMMARY

Fourteen normal and eighteen diabetic pancreases were obtained at autopsy and the insulin and zinc content of each of these determined In the pancreas of diabetics, the total amount of insulin present amounts to only one-quarter that found in the pancreas of normal individuals Likewise, the amount of zinc contained the pancreas of diabetics is only

mally present There is no marked difference in the zinc concentration in livers of diabetics and non-diabetics Certain abnormally high and low insulin values found amongst the diabetic pancreases are discussed The possibility of a part of the zinc in the pancreas being concerned with the storage of insulin is suggested

BIBLIOGRAPHY

1 Banting, F G and Best, C H, The internal secretions of the pancreas J Lab and Clin Med, 1921, 7, 251

2 Scott, D A and Fisher, A M, The effect of zinc salts on the action of insulin. J Pharmacol and Exper Therap, 1935, 55, 206

3 Scott, D A and Fisher, A M, The prolongation of insulin action by protamine and zinc. J Biol Chem (Proc.), 1936, 114, lxxxviii

4 Fisher, A M and Scott, D A, Spermine, zinc and insulin J Pharmacol and Exper Therap, 1937, 61, 21

5 Fisher, A M and Scott, D A, The effect of various substances on the action of insulin. J Pharmacol. and Exper Therap, 1936, 58, 93

6 Bischoff, F and Maxwell, L C, Influence of delayed resorption of the protein hormones J Biol Chem. (Proc), 1936, 114, xi

7 Houssay, B A., Diabetes as a disturbance of endocrine regulation. Am J M Sc., 1937, 193, 581

8 Young, F G, Permanent experimental diabetes produced by pituitary (anterior lobe) injections Lancet, 1937, 2, 372.

9 Scott, D A., Crystalline insulin Biochem J, 1934, 28, 1592

10 Scott, D A and Fisher, A M, Crystalline insulin. Biochem J, 1935, 29, 1048

11 Scott, D A, The assay of the international crystalline insulin standard by the mouse method. Quart. Bull Health Organ, League of Nations, 1936, 5, 607

12 Fisher, A M and Scott, D A, The insulin content of the pancreas in cattle of various ages J Biol Chem, 1934, 106, 305

13 Scott, D A, and Fisher, A M, Studies on the pancreas and liver of normal and of zinc-fed cats Am J Physiol, 1938, 121, 253

THE IODINE BALANCE IN NODULAR GOITER[1] [2]

By ITALO D PUPPEL AND GEORGE M CURTIS

(From the Department of Research Surgery of the Ohio State University Columbus)

(Received for publication June 30 1938)

Patients with toxic nodular goiter present an increased urinary excretion of iodine This is greater than that observed in patients with exophthalmic goiter (1) The blood iodine and the basal metabolic rate are also increased, but to a lesser degree than in exophthalmic goiter (1) In non toxic nodular goiter, however, the urinary excretion of iodine, the blood iodine, and the basal metabolic rate are normal or may be even decreased (3)

More recently we have found that the iodine metabolism of exophthalmic goiter is greatly augmented (2) This is shown in the elevated blood iodine, the increased excretion of iodine through one or all excretory channels, and the increased negative iodine balance on a low iodine intake It consequently appeared desirable to extend balance studies to patients with nodular goiter, in order better to compare the iodine metabolism in these three thyroid diseases We have therefore determined the iodine balance of two patients with non toxic nodular goiter over a total period of 36 days and that of two patients with toxic nodular goiter over a total period of 54 days

METHODS

Our experimental and laboratory methods have been given (2, 4, 5) A constant regimen of hospital management was begun five to six days prior to investigation and then maintained throughout the period of study The daily diets were selected from a limited number of foods They were low in iodine and calcium content adequate in other respects, and as attractive as possible. They were constant for each individual during the preoperative period The iodine content of the food as actually served to each patient was determined (2)

Of necessity, the diets were changed in the immediate postoperative period The patient was eventually operated on the first day of a period An aliquot part of the food eaten during the first five postoperative days was analyzed for its iodine content The total food iodine thus determined was equally divided for the period (three-day) of operation and for the first postoperative (three day) period The constant diet used preoperatively was resumed in the later postoperative periods The water ingested was single distilled and iodine free. The iodine inspired by the average individual in this region has been determined as approximately 1 microgram per day, which is negligible. Methods of preparation of the diet of collection of the excreta, and of chemical analysis of the specimens have been described elsewhere (2, 4, 5)

Non toxic nodular goiter

The iodine balance of two women with nontoxic nodular goiter was determined over a total period of 36 days One showed a physiological iodine balance (Figure 1) The other revealed a tendency toward a greater retention of iodine than normal (Table I)

Protocols may be briefly presented as follows

D W Numbers 370617 and 371011

A white housewife of 23 was readmitted to the Research Surgery Service on February 22 1937 for the management of non toxic nodular goiter She had been aware of goiter for thirteen years. This had grown progressively larger but did not give rise to symptoms until about four years ago She then noted dysphagia and dyspnea There were no toxic symptoms She had received no iodine nor thyroid medication in any form. She had been under hospital observation from February 4 to February 19 but was unable to remain for the completion of our metabolic studies at that time. Physical examination showed a large nodular goiter involving both lobes and the isthmus. Roentgenographic study revealed the trachea deviated to the right and the retrotracheal space widened.

Laboratory examination showed negative Wassermann and Kahn reactions. The blood

[1] This investigation was aided by a grant from the Committee on Scientific Research of the American Medical Association.

[2] Presented before the American Society for Experimental Pathology at Baltimore, Maryland March 31 1938.

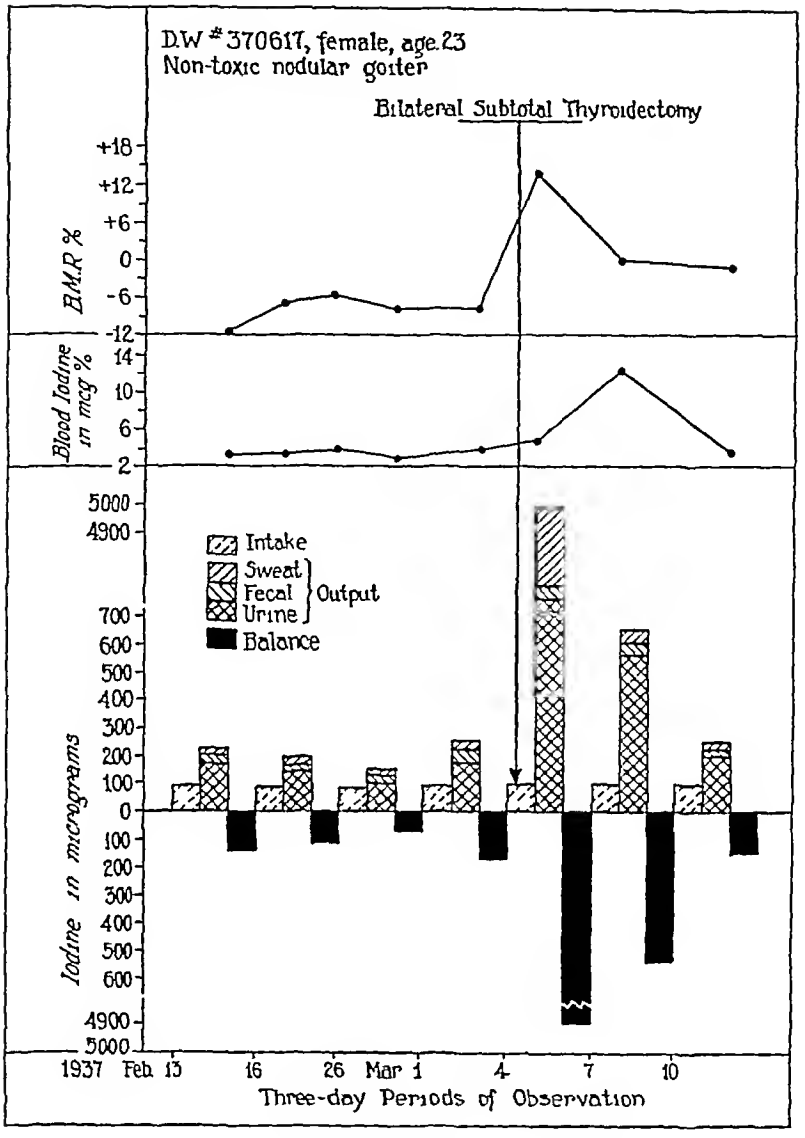


FIG 1 IODINE BALANCE OF A PATIENT WITH NON-TOXIC NODULAR GOITER

Note the continuous but normal negative iodine balance preoperatively The iodine excretion and the balance returned to within physiological limits as early as the second postoperative period.

tive save for a moderate hypochromic anemia. The urine was negative. The blood urea nitrogen was 9 mgm per cent. The phenolsulphonphthalein test for renal function was normal The basal metabolic rate on February 23 was minus 6 with the basal pulse at 57, respirations 16, temperature 98° F, blood pressure 98/56, and the body weight 115 pounds

Iodine and calcium balance studies were made from February 13 to February 19 and from February 26 to March 13 Bilateral subtotal thyroidectomy was accomplished on March 4 The postoperative course was uneventful The patient was discharged on March 13, 1937

Comments The blood iodine ranged from 2 7 to 3 6 micrograms[3] per cent, which is low normal On a low iodine intake averaging 74 micrograms per three-day period, the iodine balance remained continuously negative, but normal, and averaged 129 micrograms per three-day period over 12 days The excretion of iodine was principally through the urine (Figure 1)

Thyroidectomy was accomplished without iodine medication Immediately postoperatively there ensued a great increase in the excretion of iodine particularly through the urine. This had returned toward normal as early as the sixth day postoperatively (Figure 1)

[3] A microgram equals 0 001 mgm It is frequently called a gamma

TABLE I

B S Number 370995, female age 35 preoperative diet 2250 calories 52 grams protein non-toxic nodular goiter

Period	Date when started	Weight	Iodine						Date	Blood iodine	Basal metabolic rate	Remarks
			Output				In take	Balance				
			Urine	Feces	Sweat	Total						
	1937	*kgm*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*1937*	*micrograms per cent*	*per cent*	
I	March 1	87	63	29	31	123	92	−31	March 3	2.2	−10	Bilateral subtotal thyroidectomy March 8 1937
II	March 4	87	91	27	32	150	57	−93	March 6	2 2	−8	
III	March 7	87	2334	26	161	2521	75	−2446	March 8	2 2	−10	
IV	March 10	87	268	46	30	344	59	−285	March 10	3 1	−5	
									March 12	2 2	+12	
V	March 13	87	191	34	30	255	75	−180	March 15	1.9	+7	

The entire goiter removed weighed 290 grams. It consisted of multiple irregular colloid nodules and revealed the degenerative changes characteristic of nodular goiter The microscopic examination showed nodular colloid goiter It contained approximately 48 mgm. of iodine.

B S Number 370995

A white housewife of 35 was admitted to the Research Surgery Service on February 21 1937 for surgical treatment of non toxic nodular goiter She had been well up to five years ago when she first noted an enlarged neck. This had progressively increased in size. She first noted dyspnea and dysphagia two years ago A year later she became aware of occasional palpitation and increased nervous instability She had had no iodine nor thyroid medication in any form. Physical examination showed a bilateral nodular goiter which was partially intrathoracic.

Laboratory examination revealed negative Wassermann and Kahn reactions. The blood was normal save for a moderate hypochromic anemia. The urine was normal The phenolsulphonphthalein test for renal function was normal The blood urea nitrogen was 9 mgm. per cent. The basal metabolic rate on February 28 was minus 8 with the basal pulse 68 respiration 16 temperature 97 4° F., blood pressure 132/80 and the body weight 191 pounds.

Iodine and calcium balance studies were made from March 1 to March 16 Thyroidectomy was accomplished on March 8 The postoperative course was uneventful. The basal metabolic rate on March 14 was plus 7 with the basal pulse 69 respirations 18 temperature 97° F., blood pressure 112/78 and the body weight 187 pounds. The patient was discharged on March 16 1937

Comments The blood iodine was 2.2 micrograms per cent, which is low normal. On a low iodine intake averaging 75 micrograms per three-day period over 6 days the iodine balance remained continuously negative averaging 62 micrograms per three-day period, which is less than normal (Table I)

Bilateral subtotal thyroidectomy was accomplished without use of iodine. There was postoperatively an immediate rise in the excretion of iodine particularly through the urine and a consequent increased negative iodine balance (Table I) This was still increased when she was dismissed on March 16 (Table I)

Seventy four grams of goiter was removed. It revealed multiple, irregular colloid nodules There was evidence of fibrosis hemorrhage, calcification, and of cystic degeneration. Microscopic examination showed nodular colloid goiter It contained approximately 19 mgm. of iodine.

Toxic nodular goiter

We have determined the iodine balance of two women with toxic nodular goiter (Table II and Figure 2) over a total period of 54 days On a low iodine intake averaging 117 micrograms per three-day period over a total period of 15 days, they revealed a profound disturbance of the iodine metabolism and a continuous negative iodine balance which was from three to four times greater than normal (Figure 3)

Protocols may be briefly presented as follows

R. J Number 380303

A colored woman of 31 was admitted to the Research Surgery Service on January 15 1938, for the surgical management of hyperthyroidism. She presented the characteristic features of toxic goiter emotionalism insomnia, nervousness, tremor palpitation, moist skin loss of body weight and a rise of the basal metabolic rate. She had been well up to 1929 when she first noted a tumor in her neck. This goiter had slowly become larger However it remained asymptomatic until about a year ago when she began to note progressive increase in nervous excitability and irritability dyspnea, palpitation on exertion and excitement tremor intolerance to heat, and a loss of approximately 20 pounds of weight. She had had no iodine nor thyroid medication in any form.

TABLE II

R J, Number 380303, female, age 31, toxic nodular goiter, diet 2600 calories, 64 grams protein

Period	Date when started	Weight	Iodine						Date	Blood iodine	Basal metabolic rate	Remarks
			Output				Intake	Balance				
			Urine	Feces	Sweat	Total						
	1938	*kgm*	*micrograms*	*micrograms*	*micro grams*	*micrograms*	*micrograms*	*micrograms*	*1938*	*micrograms per cent*	*per cent*	
I	January 28	49	303	333	30	666	120	−546	January 27	9 5	+27	General Hospital Management
II	January 31	50	298	145	23	466	121	−345	January 30	8 5	+19	
III	February 3	50	293	120	44	457	120	−331	February 2	8 5	+35	
IV	February 6	50	326	101	39	466	122	−344	February 5	9 0	+24	
V	February 9		2793	185	405	3383	120	−3263	February 7	8 1		Bilateral subtotal thyroidectomy—February 9, 1938
VI	February 12		513	138	27	678	120	−558	February 10	8 1		
XI	February 27	48	302	32	16	350	110	−240	March 1	2 0	−10	
XII	March 2	48	231	29	22	282	113	−169	March 4	2 4	−10	

Physical examination showed a well-developed, well-nourished, but unusually apprehensive colored woman. There was a diffuse, symmetrical enlargement of the anterior neck with no palpable nodule formation. The trachea was not palpable. The hands and tongue showed marked tremor The exophthalmometric readings were O D 18 and O S 18 mm There was tachycardia and a soft systolic aortic murmur Roentgenograms of the neck revealed a large nodule of the left thyroid lobe.

Laboratory examination revealed negative blood Wassermann and Kahn reactions The blood examination was normal save for a slight hypochromic anemia. The urine was negative. The blood urea nitrogen on January 20 was 15 5 mgm per cent. The blood cholesterol on January 25 was 155 mgm per cent. The serum protein on February 7 was 6 4 grams per cent. The glucose tolerance was normal The bromsulphalein, galactose, and hippuric acid tests for liver function were normal. The phenolsulphonphthalein test for kidney function showed 60 per cent excretion during the first and 15 per cent during the second hour following intravenous administration The basal metabolic rate on January 18 was plus 35 with the basal pulse 90, respirations 20, temperature 98 0° F, blood pressure 136/76, and the body weight 110 pounds

Iodine and calcium balance studies were made from January 28 to March 5 She menstruated from February 8 to February 11 Thyroidectomy was accomplished on February 9 Her first four postoperative days were stormy The temperature, pulse, and respirations were as high as 104° F., 160, and 35 respectively Ten to fifteen cubic centimeters of a purulent fluid was liberated from the wound on February 13, the fourth postoperative dav Cultures showed a predominance of hemolytic streptococci, with a few nonhemolytic forms The patient improved almost immediately following drainage. Azochloramide irrigation was instituted. The postoperative course was otherwise uneventful The basal metabolic rate on March 4 was minus 10 with the basal pulse 84, respirations 18, temperature 97 8° F, blood pressure 128/88, and the body weight 104 pounds The patient was discharged on March 5, 1938

Comments The blood iodine averaged 8 9 micrograms per cent which is elevated above normal On a low iodine intake averaging 122 micrograms per three-day period, there was a great increase in the excretion of iodine particularly through the urine and feces This resulted in a greatly increased negative iodine balance which averaged 393 micrograms per three-day period (Table II)

Thyroidectomy was accomplished without iodine medication. There immediately ensued an increased excretion of iodine and an increased negative iodine balance. These returned toward normal as early as the twenty-fourth day postoperatively The blood iodine and the basal metabolic rate returned to normal (Table II) The clinical status of the patient improved

A large, irregular nodule of the left lobe of the thyroid was removed It weighed 175 grams It was covered by a thin capsule and was composed of a very friable, moist colloid tissue. There was gross evidence of edema, fibrosis, and varying degrees of vascular changes, old hemorrhage, and cholesterol deposits Microscopic examination showed nodular colloid goiter It contained approximately 28 mgm of iodine.

S W, Number 370163

A white housewife of 53 was admitted to the Research Surgery Service for the management of toxic nodular goiter on January 10, 1937 She had been aware of goiter for about 20 years However, it remained asymptomatic until about two years ago when she noted nervous instability, palpitation, and dyspnea on exertion. These symptoms had become more pronounced during the past four or five months, with an increase in the size of the goiter and an increased appetite accompanied by a weight loss of five pounds She had had no iodine therapy for three months, since mid-October Menstru-

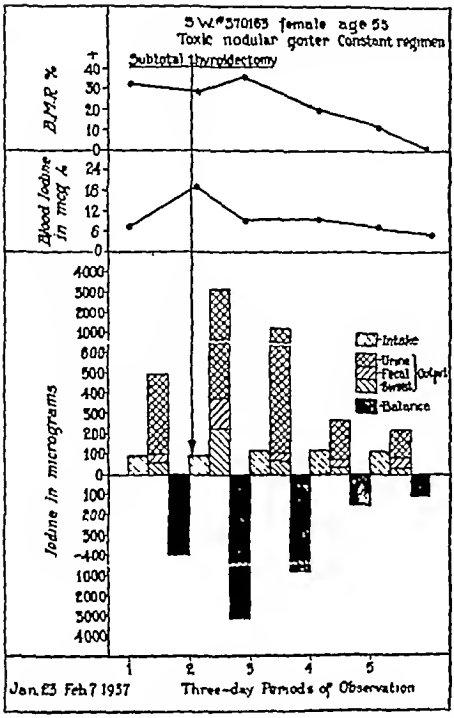


FIG. 2. IODINE BALANCE OF A PATIENT WITH TOXIC NODULAR GOITER

The iodine excretion and the negative iodine balance are typically increased preoperatively Note that as early as the second period postoperatively the basal metabolic rate and the blood iodine, as well as the excretion of iodine and the iodine balance, had returned to within normal limits.

ation ceased five months ago Physical examination revealed a bilateral nodular goiter of moderate size. There was slight tremor of the extended hand, tachycardia and a soft systolic aortic murmur The exophthalmometric readings were O D 16 and O S 16 mm.

Laboratory examination showed negative blood Wassermann and Kahn reactions. The blood and urine were normal The phenolsulphonphthalein test showed 60 per cent excretion of the dye during the first and 5 per cent during the second hour following intravenous administration. The blood urea nitrogen on January 11 was 12 mgm. per cent. The basal metabolic rate on January 22 was plus 25 with the basal pulse 109 temperature 98.4° F., respirations *14 blood pressure 166/94 and the body* weight 110 pounds.

Iodine and calcium balance studies were made from January 23 to February 7 Subtotal thyroidectomy was accomplished on January 26. The basal metabolic rate and the clinical status of the patient gradually returned to normal postoperatively The basal metabolic rate on February 7 was 0 with the basal pulse 81 temperature 98.2° F., respirations 11, blood pressure 142/88, and the body weight 105 pounds. The patient was discharged on February 7, 1937

Comments This patient with toxic nodular goiter showed an increased negative iodine balance preoperatively (Figure 2) The blood iodine was elevated, averaging 6 9 micrograms per cent. Thyroidectomy was then performed without use of iodine. There was an immediate increased negative iodine balance, principally as a result of an increased urinary excretion of iodine (Figure 2) The basal metabolic rate and the blood iodine were also elevated immediately postoperatively However the negative iodine balance returned to within normal limits during the sixth to twelfth day postoperatively The basal metabolic rate and the blood iodine also returned to within normal range.

The entire goiter removed weighed 80 grams. It was composed throughout of irregular colloid nodules. There was evidence of the characteristic degenerative changes. The microscopic examination showed nodular colloid goiter and a moderate degree of lymphocytic infiltration. It contained approximately 13 mgm. of iodine.

DISCUSSION

Three normal individuals maintained on a low iodine intake, averaging 87 micrograms per three-day period over a total period of 24 days, remained in continuous negative iodine balance which averaged 126 micrograms per three-day period (2) (Figure 3) The total excretion of iodine averaged 213 micrograms per three-day period (Figure 3) The greatest excretion was through the urine, averaging 72 per cent. Fifteen per cent was excreted through the feces and 13 per cent through the sweat (2) (Figure 3) The blood iodine averaged 4.3 micrograms per cent. These data would indicate that in normal individuals a certain amount of iodine is excreted daily over an as yet undetermined length of time. Only when iodine was furnished in excess of this amount was there established a positive iodine balance (2) There are several variants in the iodine metabolism of normal individuals (2, 6)

Two *non toxic nodular goiter patients* maintained on a low iodine intake, averaging 74 micrograms per three-day period over a total period of 18 days, showed an average negative iodine balance which was within physiological limits (Fig

FIG 3 A GRAPHIC REPRESENTATION OF THE IODINE BALANCE OF THREE NORMAL CONTROLS COMPARED WITH THAT OF TWO PATIENTS WITH NON-TOXIC NODULAR GOITER, TWO PATIENTS WITH TOXIC NODULAR GOITER, AND THREE PATIENTS WITH EXOPHTHALMIC GOITER AS SHOWN IN TABLE IV

ure 3) The total excretion of iodine averaged 180 micrograms per three-day period The greatest excretion was through the urine averaging 67 per cent Seventeen per cent was excreted through the feces and 16 per cent was excreted through the sweat However, one patient even showed a tendency for retention of iodine greater than normal (Table I) This latter confirms the findings of Scheffer and v Megay (7) The decreased total excretion resulted principally from a decreased urinary excretion of iodine (Table I) The blood iodine was low normal, averaging 3 0 micrograms per cent

Three patients with exophthalmic goiter, maintained on a low iodine intake averaging 86 micrograms per three-day period over a total period of 33 days, showed a great increase in the excretion of iodine particularly through the feces (2) (Figure 3) This resulted in an increased negative iodine balance of from two to three times the normal (Figure 3) The total excretion of iodine was 414 micrograms per three-day period The greatest excretion occurred through the urine, averaging 49 per cent Forty per cent was excreted through the feces and 11 per cent through the sweat (Figure 3) The blood iodine was increased, averaging 9 0 micrograms per cent Two other patients with exophthalmic goiter were maintained on an iodine intake sufficient to keep a normal individual in positive iodine balance These two patients also showed a negative iodine balance There are several factors which may influence the increased negative iodine balance of exophthalmic goiter The increased iodine balance returned to within normal limits subsequent to adequate thyroidectomy Increased iodine

feeding to an exophthalmic goiter patient resulted in an immediate tremendous retention of iodine and a consequent positive iodine balance which was twice that of the normal control A negative iodine balance is not necessarily characteristic of exophthalmic goiter Even a positive iodine balance can be readily maintained if the intake of iodine is sufficiently large or in excess of the increased requirements of the hyperthyroid organism (2)

Two patients with toxic nodular goiter investigated on a low iodine intake averaging 117 micrograms per three-day period over a total period of 15 days showed a great increase in the excretion of iodine particularly through the urine (Figure 3) This resulted in an increased negative iodine balance of from three to four times the normal (Figure 3) The total excretion of iodine was 510 micrograms per three-day period The greatest excretion occurred through the urine, averaging 63 per cent. Twenty nine per cent was excreted through the feces and 8 per cent through the sweat (Figure 3)

Our data would indicate, therefore, that there may be a fundamental difference in the excretion of iodine in toxic nodular and exophthalmic goiter We have recently demonstrated that in 9 patients with toxic nodular goiter there is an increased daily excretion of iodine in the urine over that of 40 patients with exophthalmic goiter who showed a much higher basal metabolic rate (3) Furthermore, our present data (Figure 3) reveal that in two patients with toxic nodular goiter and a basal metabolic rate of plus 28 there is a greater total excretion of iodine than that in three exophthalmic goiter patients with a basal metabolic rate averaging plus 40 (Figure 3) This resulted in a greater negative iodine balance than in exophthalmic goiter (Figure 3) In addition, in exophthalmic goiter the greatest increase in excretion was through the feces, in toxic nodular goiter the greatest increase was through the urine (Figure 3)

The true significance of these differences in the iodine excretion in toxic nodular and exophthalmic goiter is obscure. In an attempt to determine the nature of these differences we investigated the excretion of iodine through the various channels during and immediately following desiccated thyroid therapy to a patient with hypothyroidism (Table III) This patient showed a basal metabolic rate of approximately minus 20 immediately before administration of desiccated thyroid, four grains daily, and about five months prior to our iodine balance studies Desiccated thyroid therapy in similar dosage was continued during the first six days of investigation The basal metabolic rate was established at minus 4 There was an increased excretion of iodine through all channels but particularly through the urine However, the iodine intake was also increased by desiccated thyroid ingestion so that the iodine balance remained physiologic (Table III) The total iodine excreted averaged 1250 micrograms per three-day period The greatest excretion occurred through the urine, averaging 80 per cent. Sixteen per cent was excreted through the feces and 4 per cent through the sweat (Table

TABLE III

I M Number 366101, hypothyroid male age 34, diet 2890 calories 64 grams protein

Period	Date when started	Weight	Iodine						Date	Blood iodine	Basal metabolic rate	Remarks
			Output				In take	Balance				
			Urine	Feces	Sweat	Total						
	1936	kgm	micrograms	micrograms	micrograms	micrograms	micrograms	micrograms	1936	micrograms per cent	per cent	
I	November 17	91	910	240	52	1202	1220	+18	November 17	6 1	−4	Desiccated thyroid grains 4 daily from Nov 18 to Nov 23
II	November 20	93	1110	160	37	1307	1220	−87	November 20	5 4	−11	
III	November 23	91	413	122	36	571	68	−503	November 23	5 1	−5	
IV	November 26	91	311	68	36	415	68	−347	November 26	3 7	−7	
									November 27		−15	
V	November 29	92	142	33	25	200	68	−132	November 29	4.3	−11	
									December 1		−21	
									December 2	3 4	−19	

III) This simulated the percentage excretion of iodine through the various channels of normal individuals (2) and of patients with toxic nodular goiter rather than that of patients with exophthalmic goiter (2) Thyroid therapy was then discontinued Immediately there ensued an increased negative iodine balance which simulated that of hyperthyroidism This presumably resulted from the continued consumption of stored thyroid hormone and a consequent continued mobilization and excretion of iodine in the presence of a lessened iodine intake As the stored thyroid hormone of this hypothyroid patient was depleted, the excretion of iodine slowly decreased from 1250 to 200 micrograms per three-day period over 9 days following cessation of ingestion of desiccated thyroid (Table III) The iodine balance again returned to within physiological limits This was accompanied by a decrease in the basal metabolic rate of from minus 4 to minus 20 (Table III) The percentage excretion of iodine through the various channels did not appreciably change Seventy-three per cent was excreted through the urine, 18 per cent through the feces and 9 per cent through the sweat This again simulated that of normal individuals and that of toxic nodular goiter rather than that of exophthalmic goiter Further investigation of the nature of these differences in the excretion of iodine in these diseases of the thyroid should prove valuable

The increased negative iodine balance of hyperthyroidism may result from previous iodine feeding and the subsequent high storage of easily mobilizable iodine within the body as discussed earlier (2) However, in each instance an average of approximately 90 per cent of the goitrous tissue was removed The total iodine content of each gland removed in non-toxic nodular goiter averaged 34 mgm which is greater than that of toxic nodular goiter which averaged 20 mgm Therefore, the primary mechanism for this increased excretion of iodine in toxic nodular goiter was not caused by a higher storage of total iodine in the thyroid over that of non-toxic nodular goiter

In all instances (Figures 1 and 2) (Tables I and II) there occurred, postoperatively, a transient increase in the excretion of iodine and particularly through the urine This resulted, in part, from iodine containing catgut which was used as suture material (8) However, of greater significance is the fact that the excretion of iodine and the iodine balance had returned to normal in subsequent periods (Figures 1 and 2)

In toxic nodular goiter the clinical symptomatology had improved as early as the sixth to the twelfth day postoperatively (Patient S W) The basal metabolic rate and the blood iodine had returned to within normal limits (Figure 2) The excretion of iodine and the iodine balance had also returned to within physiologic limits (Figure 2) This would indicate that the increased excretion of iodine in toxic goiter results directly or indirectly from an overfunctioning thyroid gland

SUMMARY

1 Two non-toxic nodular goiter patients maintained on a low iodine intake showed an average negative iodine balance which was within physiological limits One patient even showed a tendency for retention of iodine over that of the normal controls The blood iodine was low normal, averaging 3 0 micrograms per cent

2 Two patients with toxic nodular goiter investigated on a low iodine intake showed a great

TABLE IV

The iodine balance in diseases of the thyroid gland A comparison of the iodine balance of normal individuals with that of nodular and exophthalmic goiter patients

Type of goiter present	Number of patients	Total days of investigation	Average basal metabolic rate	Average blood iodine	Average output per 3 day period				Average intake per 3 day period	Average balance per 3-day period
					Urine	Feces	Sweat	Total		
			per cent	*micrograms per cent*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*
1 None normal controls	3	24	−7	4 3	154	31	28	213	87	−126
2 Non-toxic nodular	2	18	−8	3 0	120	31	29	180	74	−106
3 Toxic nodular	2	15	+28	8 5	323	149	38	510	117	−393
4 Exophthalmic	3	33	+40	9 0	204	164	46	414	86	−328

increase in the excretion of iodine and particularly through the urine. This resulted in an increased negative iodine balance of from three to four times the normal. The blood iodine was increased, averaging 8 5 micrograms per cent

3 In two toxic nodular goiter patients with a basal metabolic rate of plus 28 there was a greater total excretion of iodine than that of three exophthalmic goiter patients with a basal metabolic rate of plus 40 In exophthalmic goiter the greatest increase in excretion was through the feces in toxic nodular goiter the greatest increase was through the urine

4 The percentage excretion of iodine through the various channels during and immediately following desiccated thyroid therapy to a hypothyroid patient simulated that of normal persons and that of patients with toxic nodular goiter rather than that of exophthalmic goiter

5 This increased mobilization, circulation and excretion of iodine and the profound disturbance of the iodine balance of toxic nodular goiter returned to within normal limits as early as the sixth to the twelfth day following adequate thyroidectomy

We wish to acknowledge invaluable technical assistance from Norman Matthews Ruth Bierbaum, Jerry Meyer, Arthur James, Bernard Lauer, Marinda Wickham, Freda Gooding and Norma Davis as well as the cooperation from Miss Emma McCormick and her staff of The Department of Dietetics, University Hospital.

BIBLIOGRAPHY

1 Curtis, G M., and Puppel, I. D., Increased urinary excretion of iodine in hyperthyroidism. Arch Int. Med. 1937 60 498.

2. Puppel I D, and Curtis, G M., The iodine balance in exophthalmic goiter Arch. Path. (In press)

3 Curtis, G M and Phillips, F J., The urinary excretion of iodine. I The loss of iodine in the urine following thyroidectomy J Clin. Invest., 1934 13, 777

4 Puppel, I. D., and Curtis G M., Calcium and iodine metabolism in thyroid disease. Arch. Int. Med., 1936 58, 957

5 Matthews N L. Curtis, G M and Brode, W R., The determination of iodine in biological materials. Indust. and Eng Chem., Anal Ed. (In press)

6 Curtis, G M Puppel I. D Coic, V V., and Matthews N L., The normal urinary iodine of man. J Lab and Clin. Med 1937, 22, 1014

7 Scheffer L., and v Megay, L., Jodstoffwechsel bei Kropftragern. Klin. Wchnschr., 1935 14, 1360

8. Puppel, I D, and Curtis, G. M., Unpublished data.

THE USE OF THE SKIN TEST WITH THE TYPE SPECIFIC POLYSACCHARIDES IN THE CONTROL OF SERUM DOSAGE IN PNEUMOCOCCAL PNEUMONIA

By COLIN M. MacLEOD CHARLES L. HOAGLAND and PAUL B BEESON

(From the Hospital of The Rockefeller Institute for Medical Research New York City)

(Received for publication July 1 1938)

The use of the skin test with the specific capsular polysaccharide in determining the amount of serum necessary for the treatment of Type I pneumococcal pneumonia was described by Francis in 1933 (1) A series of 53 cases was reported, 48 of whom were treated with Type I unconcentrated antipneumococcal horse serum In all but 1 of the 46 recovered cases, a positive reaction was obtained at about the time of recovery, and in 7 fatal cases the skin tests were consistently negative. Francis pointed out that a positive skin test was invariably associated with the presence of circulating type specific antibody but that, in addition, reactive tissues are necessary, since in the cases who died, even though antibody was present in the blood, the skin test remained negative

In the treatment of Type I and Type II pneumonia with concentrated antipneumococcal horse serum Finland and Sutliff (2) reported that 20 of the 23 patients who recovered gave positive skin reactions Of 5 treated patients who died, the reaction remained negative in all but one

Abernethy (3) discussed the value of the skin test as a guide in the control of dosage of concentrated antipneumococcal horse serum in his report on 25 cases of Type I pneumonia and stressed its importance in determining the minimum amount of serum necessary for the treatment of a given case.

The present communication deals with the use of the skin test in the control of dosage of Types I and II concentrated antipneumococcal horse serum, and of unconcentrated antipneumococcal rabbit serum Types I, II, III, V, VII, and VIII [1]

[1] Throughout this paper concentrated antipneumococcal horse serum and unconcentrated antipneumococcal rabbit serum will be referred to respectively as horse serum and rabbit serum

MATERIALS AND METHODS

The study was carried out on patients with pneumococcal pneumonia admitted to the Hospital of The Rockefeller Institute. The Type I and Type II concentrated antipneumococcal horse serum employed in therapy was obtained through the courtesy of Dr Augustus B Wadsworth of the New York State Department of Health The unconcentrated antipneumococcal rabbit serum was prepared in this laboratory according to the method of Goodner, Horsfall, and Dubos (4) All serum used was monovalent, and was given intravenously

Specific polysaccharides Protein free specific polysaccharides of Pneumococcus Types I, II, III and VIII were prepared by the methods employed in this laboratory (5), and were free of the species specific "C substance." The Type V polysaccharide was prepared by a modification of the Sevag method (6) The Type VII polysaccharide was a commercial preparation in which the species specific "C substance" was present as an impurity

The polysaccharides were dissolved in physiological salt solution The saline was freshly prepared from water doubly distilled in glass and immediately sterilized It was known not to produce an erythematous reaction in the normal skin Sterile stock solutions of the various polysaccharides containing 1 0 mgm per cc. were stored without preservative in rubber stoppered glass vials in the ice box. In this form the preparations have been found to retain their activity for as long as 2 years Immediately before use the stock solution was diluted tenfold with saline to make a final concentration of 1 10,000 of the specific substance.

Skin tests Five hundredths to one tenth cc. of the 1 10 000 solution (0 005 to 0 01 mgm) was used for intradermal injection on the volar sur-

face of the forearm A corresponding control injection of physiological saline was always made

Tests were done before administering serum in order to assess the reactivity of the patient's skin, since false positive reactions are occasionally encountered In patients to whom serum was given in divided doses, the tests were repeated frequently during the course of treatment, and in the other cases in which the total amount of serum given was administered at a single injection, the skin tests were done at short intervals following therapy If false positive reactions were obtained with one preparation, other preparations of both homologous and heterologous polysaccharides were generally used to check the reaction

Skin tests were read after 15 minutes, and again after 30 minutes A positive reaction was defined as consisting of a firm, edematous wheal almost invariably showing pseudopodia extending outward from its border and surrounded by an erythema If there was any doubt as to whether or not a reaction was positive, more serum was given This has proved to be a good practical rule, since an unequivocally positive and specific reaction was almost always obtained after the administration of more serum

Immediately following the administration of serum, a commonly observed phenomenon was the transient "lighting-up" of a reaction at the site of previously negative tests In these instances the earlier test had been done usually within two hours before the provocative dose of serum had been given Generally the occurrence of this phenomenon indicated that sufficient serum had been given, but not always, since tests performed subsequently would occasionally be negative and more serum would then have to be given

The present paper includes the data obtained in the study of 104 patients who were tested intradermally with various type specific polysaccharides before, during, and after serum treatment In the present study stress is laid on the results of the initial skin tests done before the initiation of serum therapy since it had been noted that an occasional patient shows a positive reaction to the homologous polysaccharide even though type specific antibody is not demonstrable in the blood, and the disease is advancing Under these conditions, if the patient's skin was found to be reactive to the polysaccharide before administration of serum, it is obvious that the test could not be used as a guide to therapy and under these circumstances serum dosage had to be judged by the general clinical criteria of recovery

Patients showing a positive skin test before serum treatment In Table I it will be seen that

TABLE I

Incidence of positive and negative skin tests before serum therapy

Type of pneumonia	Number of patients	Number showing positive test before serum	Number showing negative test before serum
Type I	60	11	49
Type II	17	2	15
Type III	16	0	16
Type V	1	0	1
Type VII	3	0	3
Type VIII	7	0	7
Totals	104	13 (12 5 per cent)	91 (87 5 per cent)

of a total of 104 patients, 13 or 12 5 per cent showed a positive skin test before serum had been given, that is, at a time when the disease was still progressive In 4 of these patients determination of circulating type specific antibody showed that specific agglutinins were not present in the blood before serum treatment All of these patients responded satisfactorily to serum therapy and all recovered In each instance the skin test which was initially positive remained so throughout the course of the disease, and in convalescence None of these cases gave a history of a previous attack of pneumonia or of known infection with pneumococcus, and in only one was there a history of cutaneous hypersensitivity This patient was allergic to a wide variety of agents, and suffered from severe eczema In the remaining 12 patients no reason has been found to account for the presence of a positive skin test while the disease was at its height and before the administration of serum

Patients showing a negative skin test before serum treatment The results in this group of cases are shown in Table II In 91 (87 5 per cent) of the 104 patients the skin test was negative before the administration of serum In one patient with Type I pneumonia the skin test re-

TABLE II

Results of skin tests after serum therapy in patients showing a negative test before treatment

Type of pneumonia	Number of patients	Results of tests in patients who recovered		Results of tests in patients who died	
		Positive	Negative	Positive	Negative
Type I	49	47	1		1
Type II	15	11		1*	3
Type III	16	11		4†	1
Type V	1	1			
Type VII	3	3			
Type VIII	7	7			
Totals	91	80	1	5	5

* Died of a vascular accident 6 weeks after admission
† In 3 of these patients the skin test became negative before death

mained negative throughout the course of the disease and in convalescence, even though dramatic curative effect was obtained from the administration of Type I rabbit serum The reason for the failure of the skin to react under these favorable circumstances is unknown.

Ten of the patients in this group died In 5 of the fatal cases the skin test was negative throughout the course of the acute illness, despite the demonstration of antibody in the blood The failure of the skin to react in these cases supports the view of Francis (1) that tissue re activity, as well as free type specific antibody, is necessary in order for a positive skin reaction to occur

Five of the patients who died showed a positive skin test after serum administration In 3 of these the skin test became negative before death occurred, the other 2 patients died suddenly, and the reactivity of the skin at the time of death was not determined In 3 of the 5 fatal cases in whom a positive reaction became negative before death, the presence of specific agglutinins in blood which was obtained postmortem showed that adequate serum had been given and that the loss of skin reactivity was not caused by a lack of humoral antibody Determination of agglu tinins was not performed in the other two cases

From consideration of the results in patients showing a negative reaction before serum therapy, it will be seen that the greatest value of the test is in those cases in which the specific action of the immune serum is rendered effective by an adequate cellular response on the part of the patient, for in these the development of a positive test serves as a measure of the optimum amount of serum to be given In fatal cases the results are less clear-cut, since in such patients the ability to react may not be present or if present may subsequently disappear, even though an excess of antibody is present in the circulating blood

In 80 patients (77 per cent) out of a total of 104, the skin test was considered entirely satisfactory and served as an aid in determining when the optimum amount of serum had been administered.

Although the greater part of the experience has been obtained with Type I and Type II pneumonia, preliminary results in the disease caused by Pneumococcus Types III, V, VII, and VIII indicate that the usefulness of the test applies equally well to the control of dosage in these types

RESULTS IN VARIOUS TYPES OF PNEUMONIA

Type I pneumonia. Sixty cases have been studied, 32 of whom were treated with Type I horse serum and 28 with Type I rabbit serum. In this series of treated cases only one death occurred. The patient was admitted on the seventh day of disease suffering from Type I meningitis In this instance, the skin test with the homologous polysaccharide was negative throughout, although the patient's serum contained agglutinins for Type I pneumococcus following serum treatment Of the recovered cases only one showed a negative skin test after effective serum treatment.

Eleven patients showed a positive reaction to Type I polysaccharide before the administration of serum and in these the skin test could not be used as a guide to serum dosage In 4 of this group who were so tested, circulating Type I agglutinins were not present before serum therapy was begun

In the remaining 47 cases, the skin test which was initially negative became positive during treatment At the appearance of a positive reaction specific therapy was discontinued and recovery promptly ensued

Type II pneumonia Seventeen cases were studied, of whom 9 were treated with Type II horse serum and 8 with rabbit antiserum The

skin reaction was positive, before the administration of serum, in 2 patients with advancing pneumonia For the reasons already stated the skin test was not applicable as a guide to serum therapy in these cases

Of the remaining 15 patients, 12 developed a positive reaction in the course of serum therapy One of these patients died of a ruptured aortic aneurysm 6 weeks after admission In the 3 other fatal cases, consistently negative reactions were obtained throughout the course of the disease

Type III pneumonia All of the 16 patients who were studied were treated with Type III rabbit serum In all cases the skin test was negative before serum treatment Fifteen patients developed a positive reaction following serum, and in one fatal case the reaction was negative throughout the course of the disease

Of the 15 patients who developed a positive skin test after serum there were 4 who died The first of these, a 40-year-old female, developed a severe purpuric reaction at the site of the skin tests, purpura appearing about 12 hours after the positive skin reaction had faded, the skin test became negative before death The second patient was a female of 68 whose blood became sterile and who developed a positive skin test following serum therapy Peripheral circulatory collapse supervened, and the skin test became negative and remained so until death The third patient, a female of 62 years, died of multiple abscesses in the consolidated portions of the lungs and in both kidneys, from all of which lesions, Type III pneumococci were isolated at autopsy The blood became sterile following serum therapy associated with the development of a positive skin test The skin test again became negative, however, 48 hours before death The fourth patient, a female of 64 years who gave a history of intermittent "cardiac irregularity" of 25 years standing, developed a positive skin reaction to the Type III polysaccharide following serum, but died suddenly of pulmonary edema, a skin test was not done within 12 hours before death

In the first, second, and third patients the presence of circulating Type III agglutinins was demonstrated in association with the positive skin test, and in these cases the blood at the time of death was shown to contain specific agglutinins in high titer Determination of agglutinins was not made in the fourth case

The loss of skin reactivity in patients in whom positive reactions occurred following the use of serum has not been observed in pneumonia other than that resulting from Pneumococcus Type III

Type V and Type VII pneumonia This group comprises 4 patients only, one case of Type V pneumonia and 3 of Type VII pneumonia, all of whom were treated with the immune rabbit serum of the corresponding type In all cases the skin test with the homologous polysaccharide was negative before serum therapy, but became positive after an amount of serum sufficient to control the infection had been given There were no deaths in this group

The preparation of Type VII polysaccharide used, as previously pointed out, was contaminated by the species specific "C substance" of the pneumococcus, so that skin reactions to this material were obtained also As has been described by Francis and Abernethy (7, 8) the reaction with the "C substance" shows certain differences from that obtained with the type specific polysaccharides of the pneumococcus However, since the primary reaction with "C substance" shows only a quantitative difference from the reaction with the specific polysaccharide, the two reactions can be distinguished only with difficulty It is essential, therefore, that the test substances used should be as free as possible from extraneous impurities, otherwise the issue becomes confused and the reactions difficult of interpretation

Type VIII pneumonia The 7 patients in this group were treated with Type VIII rabbit serum None showed a positive skin test before the administration of serum, and in each instance the test became positive after an amount of serum sufficient to control the infection had been given

DISCUSSION

The skin test with the homologous specific polysaccharide has been employed as a guide in controlling the dosage of immune serum in the treatment of pneumonia resulting from pneumococcus Types I, II, III, V, VII, and VIII The advantages of this test are the ease with which it can be done, the shortness of the time required

The skin test is of greatest value in patients who show a negative reaction before serum administration, and in whom serum is effective in initiating recovery Eighty-one patients (78 per cent) in the present series fell into this category, and in this group, with but one exception, the skin test proved to be a satisfactory and valuable aid in determining the optimum amount of serum necessary for treatment

The preparations of specific polysaccharides to be used for skin tests must be as highly purified as possible, otherwise nonspecific reactions occur which are practically indistinguishable from the reaction with the specific polysaccharides Such impurities make the specific reaction almost impossible to interpret, and destroy the value of the test

BIBLIOGRAPHY

1 Francis, T, Jr, The value of the skin test with type-specific capsular polysaccharide in the serum treatment of Type I pneumococcus pneumonia. J Exper Med, 1933, 57, 617

2 Finland, M, and Sutliff, W D, Specific cutaneous reactions and circulating antibodies in the course of lobar pneumonia (II) J Exper Med., 1931, 54, 653

3 Abernethy, T J, Concentrated antipneumococcus serum in Type I pneumonia N Y State J Med., 1936, 36, 627

4 Goodner, K., Horsfall, F L, Jr, and Dubos, R. J, Type-specific antipneumococcic rabbit serum for therapeutic purposes J Immunol, 1937, 33, 279

5 Goebel, W F, The preparation of the type-specific polysaccharides of pneumococcus J Biol Chem., 1930, 89, 395

Avery, O T, and Goebel, W F, Chemoimmunological studies on the soluble specific substance of pneumococcus J Exper Med., 1933, 58, 731

6 Heidelberger, M, Kendall, F E., and Scherp, H. W, The specific polysaccharides of Types I, II and III pneumococcus J Exper Med, 1936, 64, 559

7 Francis, T, Jr, and Abernethy, T J, Cutaneous reactions in pneumonia to the somatic ("C") polysaccharide of pneumococcus J Clin Invest., 1934, 13, 692

8 Abernethy, T J, and Francis, T, Jr, Studies on the somatic C polysaccharide of pneumococcus J Exper Med, 1937, 65, 59

9 Finland, M, and Sutliff, W D, Specific cutaneous reactions and circulating antibodies in the course of lobar pneumonia (I) J Exper Med., 1931, 54, 637

10 Rogers, E S, and Wagner, H C, Relation between skin reactions to specific carbohydrate Type I pneumococcus and human blood groups Proc. Soc. Exper Biol and Med, 1935, 33, 249

11 Alston, J M, and Lowdon, A S R, Studies of the skin reactions to the specific soluble substances of the pneumococcus Types I and II Brit. J Exper Path, 1933, 14, 1

12 Tillett, W S, and Francis, T, Jr, Cutaneous reactions to the polysaccharides and proteins of pneumococcus in lobar pneumonia. J Exper Med, 1929, 50, 687

THE RESPONSE OF DIABETICS TO A STANDARD TEST DOSE OF INSULIN

By GERALD KLATSKIN

(From the Department of Medicine University of Rochester School of Medicine and Dentistry and the Medical Clinic of the Strong Memorial and Rochester Municipal Hospitals, Rochester N Y)

(Received for publication July 5 1938)

That some diabetics improve on high carbohydrate diets is an established fact (1, 2) The selection of suitable patients for this type of diet has, until the present, been one of trial and error Recently MacBryde (3) has made an attempt to select cases on the basis of insulin sensitivity He studied the response of a small group of diabetics to a standard test dose of insulin and concluded that they fell into two groups, the relatively insulin resistant and the relatively insulin-sensitive. The resistant group gained tolerance on high carbohydrate diets while the sensitive group did not In addition, the patients showing insulin resistance were usually older, frequently obese, often had vascular hypertension and showed little tendency to acidosis The relatively sensitive group were usually younger, often thin, had as a rule low blood pressure and were more prone to develop acidosis and coma. Although the insulin requirement of the resistant group was larger, the sensitive group were looked upon as more serious, judged by their tendency to acidosis On the basis of a somewhat different test, Himsworth (4) classified diabetics in a similar manner, but concluded from his experimental findings and clinical data that insulin sensitive patients tolerated high carbohydrate diets better than did the insulin insensitive.

Since these two investigators appear to have come to diametrically opposite conclusions in regard to the relationship between insulin sensitivity and response to high carbohydrate diets, it was felt worth while to study this problem further The purpose of the present investigation has been to study a relatively large group of diabetics with respect to their blood sugar response to a standard test of insulin, and to correlate, if possible, insulin sensitivity with their clinical characteristics and responses to diets of variable carbohydrate content.

METHOD OF STUDY

Fifty of a total of 197 patients attending the adult Diabetic Clinic of the Strong Memorial Hospital were chosen for this study Each had previously had a complete physical examination, blood count, urinalysis, and Wassermann reaction. They represented a fair cross section of the total clinic population and were arbitrarily selected from amongst those who had attended the clinic for at least five months and who had been most cooperative and regular in their attendance. None was suffering from infection at the time of the studies Cases in which the diagnosis of diabetes mellitus was at all questionable were subjected to a sugar tolerance test and were accepted only if they had typical diabetic responses (Cases 7, 25 40 45)

Each patient was subjected to an "insulin tolerance test" and classified as relatively insulin sensitive or relatively insulin resistant. His past record in our clinic and on any admission to the hospital was then studied and analyzed. These studies constitute the basis for this report. The patients had previously been followed from 5 to 123 months, an average of 42 months each and had usually been seen at monthly intervals—the severe cases more, and the mild cases less frequently

"Insulin tolerance test" Following the technique of MacBryde (3), one unit of insulin per ten pounds of body weight was administered subcutaneously in the fasting state. The test was performed in the clinic between 9 00 and 9 30 a.m., in a special room set aside for that purpose. The patients were required to sit quietly or lie down for the following three hours Venous blood specimens for sugar determinations were drawn fasting at one one and one-half, two and three hours. The fourth hour specimen was omitted for the convenience of the patients and staff This appears to be a justifiable omission since twelve of MacBryde's fifteen patients showed their maximum responses by the end of the third hour and of the remaining three, none would have fallen into another group had the fourth hour specimen been omitted. The one and one half hour specimen was included after a preliminary study revealed that a fair number of patients had minimum blood sugars at that time (Cases 20 25 26 37 45)

Weight The patients were weighed in their street clothes (minus hat and coat) at each visit to the clinic. Their height in stockinged feet was measured [illegible] sion and at approximately yearly [illegible]

weight for height, age and sex[1] was recorded from time to time. Patients who were 10 per cent or more over normal were considered overweight, those 10 per cent or more under normal were considered underweight

Diets were prescribed by the examining physician in grams of protein, fat, and carbohydrate. The diet was then calculated in terms of household measures of food by one of the dietitians permanently assigned to the Diabetic Clinic and was discussed with the patient At each visit to the clinic, the patient was required to bring in a detailed report of his food for the preceding day This was reduced to grams of protein, fat, and carbohydrate by the dietitian and recorded on the chart. With this frequent check on cooperation and understanding it was possible to correct errors and re-instruct the patients in the use of diets

Although no standard diets were used, they contained, as a rule, little fat, moderate carbohydrate, and from 0 75 to 1 0 gram of protein per kilogram of normal body weight. These were modified at frequent intervals, however, to improve control, to suit the patients' tastes and purses, and to adjust weight. An effort was made to maintain the weight normal for height and age or, preferably 10 per cent below In many cases, this was not possible because of a patient's unusual appetite or unwillingness to cooperate when on a low-caloric intake

In order to compare diets in patients of different weights, it was found necessary to resort to a common denominator The total glucose value per kilogram of body weight seemed the only logical one to choose, since the caloric values and protein-fat-carbohydrate ratios were not constant. The glucose value was calculated as 58 per cent of the protein, plus 10 per cent of the fat, plus 100 per cent of the carbohydrate, and expressed in grams per kilogram The control diet described by MacBryde (3) contained protein 1 0 gram, fat 1 7 grams, and carbohydrate 2 0 grams, or a total glucose value of 2 7 grams per kilogram His high carbohydrate diets (Tables IV and V (3)) contained 3 0 or more grams For simplicity of expression and analysis, the diets herein described containing 3 0 or more grams of total glucose per kilogram are considered "high carbohydrate," all others "low carbohydrate" diets The designation "low carbohydrate," therefore, obviously includes moderate carbohydrate diets as well This classification appears sound since it is not the purpose of this study to report on the effects of high or low carbohydrate diets *per se,* but rather to compare the effects of diets of variable carbohydrate content on single individuals and on groups

Insulin, when required, was self-administered 15 to 30 minutes before meal time. Of the patients requiring insulin, six took it once a day (before breakfast), eleven twice a day (before breakfast and supper), and two three times a day (before each meal) Patients recorded as having insulin reactions had at least one record of a reaction in their charts Any attempt to estimate the number or degree of reactions would, of course, have been futile.

Glycosuria. On admission to the clinic, patients were taught to test their urines with Benedict's qualitative solution in the usual fashion (5) This was usually done at least once a day (before breakfast) and frequently as often as three times a day The color of the reaction and the amount of precipitate was noted The examining physician recorded this as 0 to 4+ In addition, at each visit an overnight specimen of urine, voided before breakfast, was brought in and tested qualitatively for sugar and diacetic acid as a check on the patient's record.

Blood sugars In the clinic it was found impracticable to do fasting blood sugars because of the difficulties attending subsequent dietary management and insulin dosage. Therefore, all studies were done on blood taken one and one-half to three hours after breakfast. Venous blood was drawn and sugar determined by Benedict's (6) method at intervals of one to three months, depending on the severity of the disease. Blood sugars were averaged for each year and separately for the duration of each diet. In determining the average blood sugar level for a patient's total period of observation only the yearly averages were considered

Control For practical purposes, the control of diabetes is synonymous with the control of glycosuria There are some who would question this view, but since the other aspects of diabetic regulation are considered elsewhere, the term "control" has been adopted here to designate the degree to which glycosuria was restrained Patients were classified as "good" whose urines remained sugar-free at all times or showed an occasional trace of sugar The first month, during which diet and insulin were being adjusted, was excluded from consideration One case (Number 23) was considered "good" in spite of two attacks of acidosis of short duration, because of the absence of glycosuria at all other examinations over a long period. Control was considered "fair" where the patient usually showed mild glycosuria (0 to 1+) with occasional larger excretion of sugar (2 to 4+) "Poor" control was reserved for those patients who showed considerable glycosuria (2 to 4+) on most examinations It should be noted that the degree of glycosuria recorded occurred in spite of adjustments of diet and insulin

The patients were classified after a careful study of their records and before the insulin tolerance or other data had been computed, so that estimates of their condition might be unprejudiced.

Blood pressure was determined on admission and at irregular intervals thereafter with a Tycos aneroid sphygmomanometer A systolic pressure of over 150 mm Hg was considered hypertension Several patients (Cases 32, 9, 3, 12, 13, 44, 28, 27) had normal pressures on admission, but subsequently developed hypertension These have been classified as hypertensive.

Arteriosclerosis Patients were said to have arteriosclerosis when the peripheral arteries were palpably

[1] Metropolitan Life Insurance Company Tables Howe Scale Co, Rutland, Vt.

thickened or beaded and when the retinal vessels showed changes generally ascribed to arteriosclerosis.

RESULTS

The clinical and laboratory data of the fifty patients studied are presented in Tables I and II The terms "sensitive" and "resistant" are hereafter used synonymously with "relatively insulin-sensitive" and "relatively insulin resistant," respectively

Subcutaneous insulin tolerance The fasting blood sugars fell from 30 to 85 per cent during the three-hour test period (Table II) The average fall for the entire group was 60 per cent

In general, the absolute fall was proportional to the height of the fasting blood sugar (Figure 1) From the distribution of the points in this figure, there did not appear to be any tendency for the patients to fall naturally into sensitive and resistant groups For purposes of comparison they were, therefore, arbitrarily divided into two groups, as in MacBryde's (3) study, using the average percentage fall for the entire group as the dividing line Those which fell more than 60 per cent were classified as sensitive, those which fell less than 60 per cent were classified as resistant

Clinical characteristics There did not appear to be any significant difference between the sensitive and resistant groups with respect to weight, insulin requirement, controlability of glycosuria, average blood sugar, or incidence of arteriosclerosis and hypertension (Table I) The average age of the insulin sensitive group was lower than that of the insulin-resistant This was related to the fact that all four cases below the age of 21 fell into the former group As was to be expected, the sensitive group had a greater incidence of insulin reactions (Table I)

Acidosis occurred more frequently in the sensitive than in the resistant group This appeared to be related to the fact that all the juvenile diabetics were in the sensitive group Of the four patients in the entire series who were under 21 years of age, three developed acidosis on one or more occasions while of the remaining 46 cases, only two had acidosis (Tables I and II)

Relation of insulin tolerance to diet The control on high and low carbohydrate diets was compared in the insulin sensitive and insulin resistant

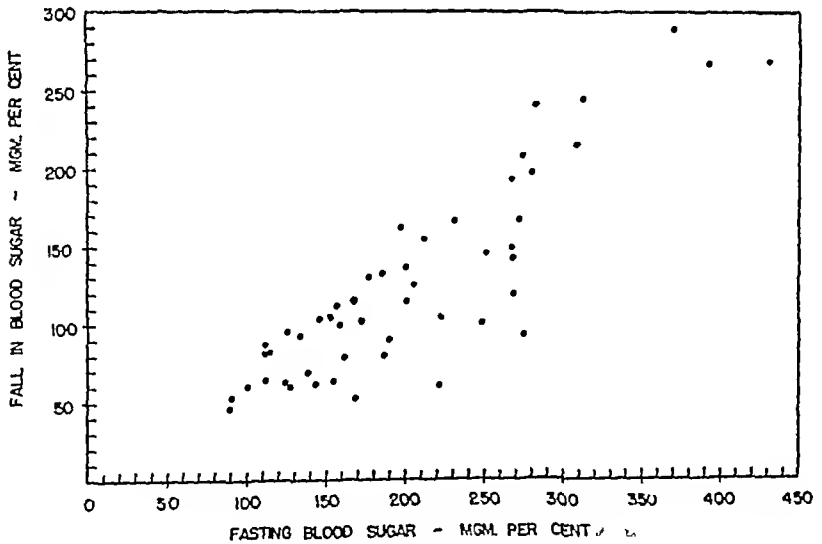


FIG. 1 INSULIN TOLERANCE TESTS

TABLE I

Summary of findings

	Insulin sensitive	Insulin-resistant
Number of cases	28	22
Sex		
Male	12	7
Female	16	15
Average age		
Years	53 2	56 9
Under 21 years	4	0
Weight		
Normal	11	8
Overweight	10	11
Underweight	7	3
Average duration of diabetes, *years*	6 4	7 2
Insulin tolerance		
Average fasting blood sugar, *mgm per cent*	217	185
Average test dose of insulin, *units*	14 6	15 9
Average fall, *per cent*	70 7	47 3
Insulin		
Average 24-hour requirement, *units*	19 1	18 8
Cases requiring		
0 units in 24 hours	9	9
1–30 units in 24 hours	13	8
31+ units in 24 hours	6	5
Reactions	12	8
Control		
Good	13	11
Fair	13	6
Poor	2	5
Average blood sugar after meals, *mgm per cent*	216 2	213 6
Complications		
Acidosis	4	1
Hypertension	10	9
Arteriosclerosis	14	12

groups The last prescribed diet in each case was used as the basis for comparison since it was presumably the optimal one under the circumstances, having usually been adjusted several times If the resistant group gains tolerance on a high carbohydrate intake, as suggested by MacBryde (3), those of the group on high carbohydrate diets should have been better controlled than those on low carbohydrate diets Also, the resistant group should have been better controlled on high carbohydrate diets than the sensitive group on similar diets No such relationship could be demonstrated (Table III)

There were fourteen cases in the series who at one time or another during their period of observation were changed from a low to a high carbohydrate diet, or *vice versa* These were

TABLE II

Clinical data

Case number	Insulin tolerance test: Blood sugar: Fasting	Insulin tolerance test: Blood sugar: Fall	Insulin tolerance test: Fall	Insulin tolerance test: Test dose	Age	Weight	Carbohydrate content of diet	Control	Hypertension	Arteriosclerosis	Acidosis
	mgm. per cent	*mgm. per cent*	*per cent*	*units*	*years*						
1	282	241	85	15	70	Normal	Low	Fair	+	+	−
2	197	162	82	20	68	Underweight	High	Good	+	+	−
3	370	289	78	16	36	Normal	Low	Fair	+	−	+
4	274	208	75	13	44	Normal	Low	Fair	−	−	−
5	211	154	73	11	68	Underweight	High	Fair	−	−	−
6	267	193	72	11	68	Underweight	High	Fair	−	+	−
7	114	82	72	13	66	Normal	High	Good	−	−	−
8	231	166	72	15	18	Overweight	Low	Fair	−	−	+
9	156	111	71	16	69	Overweight	High	Fair	+	+	−
10	308	215	70	14	20	Overweight	Low	Fair	−	−	−
11	280	197	70	13	68	Underweight	High	Good	−	+	−
12	205	125	61	15	62	Overweight	Low	Good	+	+	−
13	111	81	73	19	70	Overweight	Low	Good	+	+	−
14	185	132	71	12	56	Underweight	High	Good	+	+	−
15	177	130	73	12	62	Underweight	High	Good	−	+	−
16	312	245	79	12	55	Underweight	High	Fair	−	+	−
17	145	103	71	20	65	Overweight	Low	Good	−	−	−
18	272	167	61	14	59	Normal	Low	Fair	−	−	−
19	392	267	68	12	16	Normal	High	Poor	−	−	+
20	152	104	68	16	50	Normal	Low	Good	−	−	−
21	430	268	62	12	25	Normal	High	Poor	−	−	−
22	200	136	68	16	65	Overweight	Low	Fair	+	−	−
23	167	114	68	12	16	Normal	High	Good	−	−	+
24	111	77	69	12	55	Normal	High	Fair	−	−	−
25	100	60	60	16	59	Overweight	Low	Good	−	+	−
26	133	92	69	17	52	Overweight	High	Good	−	−	−
27	158	99	63	17	69	Normal	Low	Good	+	+	−
28	125	95	76	17	60	Overweight	Low	Fair	+	+	−
29	168	51	30	17	40	Overweight	Low	Fair	−	−	−
30	143	61	43	20	51	Overweight	Low	Good	−	+	−
31	288	119	44	14	63	Normal	Low	Poor	−	−	−
32	126	59	47	15	59	Normal	High	Poor	+	+	−
33	222	104	47	19	45	Overweight	Low	Poor	+	−	−
34	161	78	48	20	52	Overweight	Low	Fair	−	−	−
35	138	68	49	20	57	Overweight	Low	Good	+	−	+
36	124	62	50	16	36	Overweight	Low	Good	−	−	−
37	89	46	52	13	60	Underweight	High	Good	−	+	−
38	267	149	56	18	68	Overweight	Low	Good	+	+	−
39	250	145	58	14	75	Normal	High	Good	−	+	−
40	90	53	59	16	48	Normal	Low	Good	−	−	−
41	172	101	59	15	67	Normal	Low	Poor	−	−	−
42	274	93	34	16	54	Overweight	Low	Fair	+	+	−
43	189	89	47	12	46	Normal	Low	Good	−	+	−
44	267	142	53	16	62	Overweight	Low	Fair	+	+	−
45	111	64	58	14	76	Underweight	Low	Good	+	+	−
46	248	100	40	16	59	Normal	Low	Good	+	−	−
47	221	60	27	16	73	Overweight	Low	Poor	−	+	−
48	186	79	42	14	57	Normal	High	Fair	+	−	−
49	200	114	57	13	52	Underweight	Low	Fair	−	−	−
50	154	63	41	16	54	Overweight	Low	Good	−	−	−

TABLE III

The relationship between diet and diabetic control

Control	Insulin sensitive group		Insulin-resistant group	
	High carbohydrate diets	Low carbohydrate diets	High carbohydrate diets	Low carbohydrate diets
Good	7	6	2	9
Fair	5	8	1	5
Poor	2	0	1	4

studied in detail with respect to changes in glycosuria, blood sugar and 24 hour insulin requirement (Table IV) If the resistant group gains tolerance on high carbohydrate intake, a change from low to high carbohydrate diet in a resistant patient should have resulted in improvement, whereas in a sensitive patient either no change or an aggravation of his condition should have occurred No such correlation could be demonstrated Some patients gained and others lost tolerance on high carbohydrate diets quite without relation to their insulin sensitivity (Table V)

TABLE IV

Effect of changing from low to high carbohydrate diet

Case number	Dietary total glucose per kgm	On diet	Average 24-hour insulin requirement	Average p.c. blood sugar	Glycosuria
	grams	*months*	*units*	*mgm per cent*	
	INSULIN SENSITIVE GROUP				
3	2 2 3 7	2 2	44 30	208 245	No change
6	2 5 3 1	4 12	5 10	150 140	Increased
7	2 9 3.3	3 5 '	35 10	109 96	No change
8	2.3 3.2	11 1	52 34	334 257	No change
9	2.3 3.3	61 3	6 28	197 110	Increased
10	1 7 3 4	9 7	37 43	194 243	Decreased
11	2 9 3.3	9 18	33 32	240 297	No change
15	2.5 3 1	0.5 59	13 23	338 217	Decreased
18	1 4 3 0	66 1	9 0	286 258	No change
21	2 3 4 0	22 1	30 38	156 229	No change
	INSULIN RESISTANT GROUP				
32	1 8 3 0	10 3	7 53	137 294	Increased
38	2 1 3.2	4 6	18 0		Decreased
39	2 1 3 8	12 46	21 21	196 245	No change
48	2 0 3 2	3 28	0 16	215 260	Increased

TABLE V

Effect of changing from low to high carbohydrate diet

	Insulin sensitive group	Insulin-resistant group
Glycosuria		
Increased	2	2
Decreased	2	1
Unchanged	6	1
Average p c. blood sugar		
Increased	4	3*
Decreased	6	0
Average 24-hour insulin requirement		
Increased	5	2
Decreased	5	1
Unchanged	0	1

* No blood sugar values obtained in Case 38.

COMMENT

Fifty cases of diabetes mellitus were studied with respect to their response to a standard test dose of insulin In general, the absolute fall in blood sugar was proportional to the height of the fasting level, a finding first noted by Radoslav (7) Although the percentage blood sugar fall varied widely, between 30 and 85 per cent, the distribution of cases was such that no natural cleavage between insulin sensitive and insulin-resistant groups could be made out The division of diabetics into two such groups on the basis of a standard test dose of insulin appeared therefore, to be an arbitrary one When the cases were divided in that manner, using the average percentage blood sugar fall for the entire series as the dividing line, no appreciable difference in clinical characteristics or response to high carbohydrate diets could be made out between the sensitive and resistant groups The lower average age and the greater incidence of acidosis in the sensitive group were related to the fact that all four juvenile diabetics fell into that group The greater tendency of juvenile diabetics to acidosis is a well known fact (8) Whether there was any significance in their all having fallen into the sensitive group cannot be determined from the available data A much larger group of juveniles would have to be investigated before any definite conclusions could be drawn

There has been a growing conviction among students in this field (4, 9, 10) that there are extra-pancreatic factors operating in certain diabetics In some instances the operation of such factors, for example, liver disease (11, 12), thyrotoxicosis (13), and pituitary disease (14) can be clearly demonstrated In others, in whom there is simply a resistance to test doses of insulin, extra-pancreatic factors have been assumed (4, 9, 10) In the present state of our knowledge such assumptions do not appear to be justified Himsworth's (4) demonstration of the inability of "insensitive-diabetics" to transfer sugar from the blood to the tissues under the influence of insulin is highly suggestive of such a factor On the other hand, the available data on the significance of the response to a standard test dose of insulin do not warrant any conclusions regarding the pathogenesis of diabetes A study of the response to a standard test dose of insulin in normals and in diabetics with known extra-pancreatic influences at work might shed further light on the significance of the insulin tolerance test

CONCLUSIONS

(1) In diabetics the response of the fasting blood sugar to a standard test dose of insulin varies greatly

(2) The division of diabetics into relatively insulin-sensitive and relatively insulin-resistant groups is an artificial one

(3) There does not appear to be any significant relationship between the insulin-sensitivity of diabetics and their clinical characteristics or their responses to high carbohydrate diets

BIBLIOGRAPHY

1 Himsworth, H P, High carbohydrate diets and insulin efficiency Brit. M J, 1934, 2, 57
2 Geyelin, H R, The treatment of diabetes with insulin (after ten years), contrasting the effects of normal and of the older diabetic diets J A M A, 1935, 104, 1203
3 MacBryde, C M, Response to insulin as an index to the dietary management of diabetes J Clin Invest, 1936, 15, 577
4 Himsworth, H P, Diabetes mellitus its differentiation into insulin-sensitive and insulin-insensitive types Lancet, 1936, 1, 127
5 Benedict, S R., The detection and estimation of glucose in urine J A M A, 1911, 57, 1193
6 Benedict, S R., The determination of blood sugar J Biol Chem., 1925, 64, 207
7 Radoslav, C St., Über die Wirkung des Insulins auf den Blutzucker beim Menschen. Wien. Arch f inn Med, 1924, 8, 395
8 Joslin, E. P, Root, H F, White, P, Jordan, W R., and Hunt, H M, Diabetic coma. M Clin. North America 1932, 15, 829
9 MacBryde, C M, Insulin resistance in diabetes mellitus Arch Int. Med, 1933, 52, 932
10 Falta, W and Boller, R., Insulärer und insulinresistenter diabetes Klin Wchnschr, 1931, 10, 438
11 Root, H F, Insulin resistance and bronze diabetes New England J Med., 1929, 201, 201
12 Engel, R, Insulinrefraktärer Diabetes bei schwerem Leberschaden. Klin. Wchnschr, 1934, 13, 1682
13 Rosenberg, M and Meyer, W B, Klinischer Beitrag zur Pathogenese des extrainsulären "Diabetes" Deutsche med. Wchnschr., 1925, 51, 935
14 Altshuler, S S and Gould, S E., Diabetes refractory to insulin, with report of a case. Ann. Int Med., 1936, 9, 1595

THE DISTRIBUTION OF ASCORBIC ACID BETWEEN CELLS AND SERUM IN RELATION TO ITS URINARY EXCRETION

By MARTIN HEINEMANN[1]

(From the Department of Internal Medicine Yale University School of Medicine New Haven)

(Received for publication July 14 1938)

Determinations of the concentration of ascorbic acid in whole blood (1, 2, 3, 4, 5, 6, 7, 8) and in plasma or serum (9, 10, 11, 12, 13, 14, 15, 16, 17, 18) as well are believed to indicate the degree of saturation of the organism No strict correlation, however, has been established between the ascorbic acid content of whole blood and of plasma, although the concentration in cells is consistently higher than in plasma under ordinary conditions (5) The present investigation deals with the distribution of ascorbic acid in blood before and during absorption of test doses of ascorbic acid, the relationship of its concentration in whole blood and in serum to the amounts eliminated in urine. The distribution between cells and serum of ascorbic acid added to blood *in vitro* has also been observed

METHODS

Ascorbic acid was determined according to the method of Emmerie and van Eekelen (1 19 20) Instead of oxalated blood blood defibrinated by stirring with a glass rod was used in order to prevent hemolysis, which influences the content of ascorbic acid in serum or plasma In the absence of hemolysis no significant nor consistent differences could be detected between oxalated or defibrinated samples of the same blood.

By this method, ascorbic acid in blood is determined by titration against 2,6-dichlorophenol indophenol after deproteinization with trichloroacetic acid and removal of interfering substances by precipitation with mercuric acetate. Since this method has not been described in the American literature some details are given here.

Reagents

Trichloroacetic acid 10 per cent

Mercuric acetate, 20 per cent prepared according to the directions given in a recent publication (23)

Solid calcium carbonate

Procedure In whole blood ascorbic acid has been found to be fairly stable, since it appears to be protected against irreversible oxidation by the red cells (21 22) An interval of a few hours between the collection of blood and the determination of ascorbic acid therefore, is irrelevant. Ten cc. of defibrinated whole blood and an equal volume of 10 per cent trichloroacetic acid are mixed thoroughly in a 50 cc. round bottomed centrifuge tube by stirring with a glass rod thereafter one-half the volume (5 cc.) of 20 per cent mercuric acetate is added and also mixed well The mixture is neutralized with $CaCO_3$ with Congo red paper as an indicator, and immediately centrifuged for about 2 minutes. The supernatant fluid is then filtered off The procedure from this point (treatment with H_2S which is removed the next day by nitrogen and titration with 2,6-dichlorophenol indophenol) is similar to that recently described for urine (23) Scarborough and Stewart (24) observed that ascorbic acid, as determined by this method, increased if H_2S was removed one or two days later In view of these observations it must be mentioned that our specimens have been treated with nitrogen regularly between 17 and 21 hours after treatment with H_2S The time elapsing between deproteinization with trichloroacetic acid and treatment of the filtrate with H_2S should not exceed 10 minutes otherwise irreversible oxidation of the vitamin takes place. Loss of time can be reduced by preparing for the different manipulations beforehand and by using a centrifuge equipped with a brake. The quality of $CaCO_3$ has been found to be of importance some brands may contain reducing substances which are not precipitated by mercuric acetate, giving a blank reading equivalent to 6 mgm of ascorbic acid per 1000 cc. With the brand used Mallinckrodt's analytical reagent, the blank proved to be zero if high blanks are obtained, the $CaCO_3$ should be suspected.

By this method ascorbic acid, added in amounts from 5 to 10 mgm per liter to whole blood, has been recovered with a maximum error of 10 per cent in 8 experiments.

Providing that food rich in vitamin C is avoided, only slight fluctuations of ascorbic acid (from 13 1 to 14.8 mgm. per liter) were observed in the blood of a single subject examined 13 times in the course of 30 hours

Serum In plasma or serum, ascorbic acid is less stable than in whole blood (10 13 21 22), therefore these fluids have been analyzed *immediately after* separation from the cells The procedure is similar to that for whole blood but less trichloroacetic acid is required for deproteinization. To 10 cc. of serum, 5 cc. of 10 per cent trichloroacetic acid and 5 cc. of mercuric acetate are added. When only 8 or 6 cc. of serum are available, the quantities of reagents are reduced proportionally but the final volume is made up to 20 cc. by addition of distilled water in order to yield two 5 cc. aliquots for titration. This addition of distilled water after neutralization with $CaCO_3$ and before centrifuging does not influence the experimental results

[1] Foreign Physicians' Committee Fellow

In urine Ascorbic acid has been determined according to the method recently described in full detail (23) For the purpose of observing the *excretion of large amounts of ascorbic acid by saturated normal subjects following a test dose* direct titration of freshly voided urine is satisfactory

Blood cell volumes have been measured by the hematocrit method described by Eisenman *et al* (25)

In additional experiments *in vitro* ascorbic acid was not added directly to whole blood, but was dissolved in serum first to avoid hemolysis No hemolysis occurred nor were changes of cell volume noticed during the experimental period of 5 hours, providing that the amounts added did not exceed 6 mgm per liter After addition of ascorbic acid the blood was gently shaken mechanically for 4 to 5 hours at 23° C in sealed tubes of pyrex glass Crystalline ascorbic acid has been used.[2]

RESULTS

I Relationship between the concentrations of ascorbic acid in whole blood and in serum

Figure 1 shows that in subjects who have not received vitamin C for about 12 hours the concentrations of ascorbic acid in serum are only roughly correlated with those in whole blood and that the concentration is always higher in whole blood than in serum

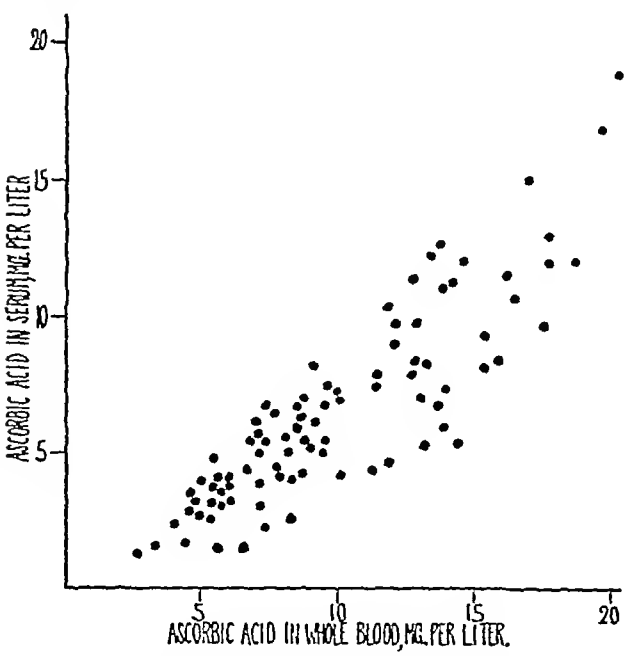


FIG 1 RELATION BETWEEN ASCORBIC ACID IN WHOLE BLOOD AND IN SERUM IN PERSONS WHO HAD RECEIVED NO VITAMIN C FOR AT LEAST 12 HOURS

New data, presented in Figure 2, confirm the almost linear relationship described by van Eekelen *et al* (3) between the concentration of ascorbic acid in whole blood and the amount needed for saturation, which has been defined in a previous communication (26)

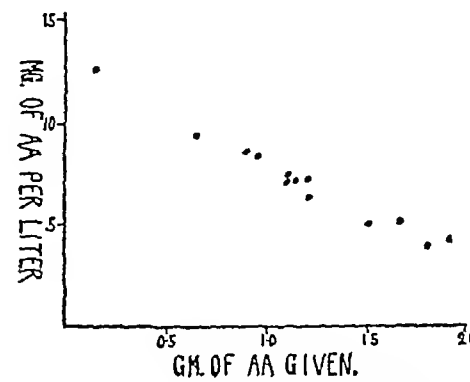


FIG 2 RELATION BETWEEN THE CONCENTRATION OF ASCORBIC ACID IN WHOLE BLOOD AND THE AMOUNTS NEEDED FOR SATURATION

From this it follows that, for the practical estimation of the degree of saturation or unsaturation of an individual, analyses of whole blood are preferable to that of serum This is further evidenced by the following reactions of a patient With 13 0 mgm per liter of whole blood and 8 3 mgm per liter of serum, she excreted 24 mgm of ascorbic acid in the urine in 6 hours following a dose of 300 mgm of ascorbic acid *per os*, while one week later, with 14 2 mgm per liter in the whole blood and only 5 3 mgm per liter in the serum, she excreted 46 mgm in the same period after the same dose This patient bled severely between the first and the second experiment Following several blood transfusions, the serum ascorbic acid of a patient with secondary anemia rose from 3 9 mgm per liter to 6.2 mgm per liter, while the whole blood ascorbic acid remained unchanged (7 1 and 7 0 mgm per liter respectively) These observations illustrate that striking exceptions from the general correlation between whole blood and serum concentrations can occur

That the concentration in serum may rise above that of whole blood after administration of ascorbic acid is apparent from Table I which pre-

[2] For the *in vitro* experiments and for the *in vivo* studies as well, ascorbic acid was supplied through the courtesy of Hoffman-LaRoche, Inc., Nutley, N J

TABLE I

Milligrams of ascorbic acid per liter of whole blood and serum at intervals after taking ascorbic acid by mouth

Intake		Before	Hours after Intake						
			1	1½	2	3	4	6	24
mgm per kgm.									
3 0	Blood	14 4	15 6		17 4	19 0	19 3	17 4	
	Serum	14 0	16 7		19.5	21.2	20 1	18 9	
15 0	Blood	4 8		6 0		9 8			6 8
	Serum	2 7		5.3		12 0			5.5

sents data from two experiments typical of 13 in which doses from 120 to 1100 mgm of ascorbic acid were taken. Absorption of quantities that cause considerable increase of blood ascorbic acid does not change the cell volume. The concentration of ascorbic acid in serum first rises above that in whole blood but reaches a peak and begins to fall while the concentration in whole blood still continues to rise (see also Figure 4)

Calculations of concentrations of ascorbic acid in blood cells

In the experiments in which cell volume measurements are available, the concentrations of ascorbic acid per liter of cells have been calculated Figure 3 presents the relationship between ascorbic acid concentrations in cells and in serum in fasting blood

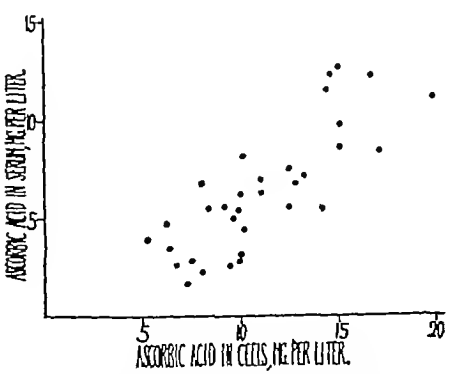


FIG. 3 RELATION BETWEEN ASCORBIC ACID IN CELLS AND IN SERUM IN PERSONS WHO HAD RECEIVED NO VITAMIN C FOR AT LEAST 12 HOURS

TABLE II

The amounts of ascorbic acid in cells and serum after taking ascorbic acid by mouth

Intake	Time after intake	Cell volume	Ascorbic acid		
			Whole blood	Serum	Cells
mgm per kgm	hours	per cent	mgm per liter	mgm per liter	mgm per liter
2	3	50	20.3	22 8	17 8
2	3	48	16.3	18.3	14 2
2	5	38	14 4	15 9	11 8
2	3	38	15.3	17 7	11.3
15 5	0	45	4 8	2 7	7.3
	1½	45	6 0	5.3	6 9
	3	45	9 8	12 0	7 1
10 0	0	43	9 4	6 8	12 8
	2	43	11 7	14.3	8 1
12 0	0	43	9 8	7.2	13 3
	2	44	18 1	25.5	8 6
8 0	0	43	8 7	5 5	13 0
4 0	1½				
5 0	3	43	14 1	17.5	9.5
	6	43	16.3	17.5	14 7
3 5	0	39	13 2	12 3	14 6
	1½	39	14.3	15.2	12 8
	2½	39	15 0	17.5	11 0
	3½	39	17 6	20 1	13 6
	5	39	18 1	18.5	17 4
2	2½	39	16 4	20 0	10 8
	4	39	16 0	20 0	9 7
	5	39	18 6	20 0	16 4
	6½	39	17 6	18.2	16 7
2	3	38	15 1	16 8	12 4
	6	38	14 9	15 9	13 2
3 5	0	36	14 4	14 0	15 0
	1	36	15 6	16 7	13 6
	2	36	17 4	19.5	13 6
	3	36	19 0	21 2	15 0
	4½	36	19.3	20 1	17 8
10 0	0	36	4.5	3 5	6.3
	1½	36	6 6	6 9	6 1
	3	37	8 7	8.3	9.5
	4½	36	9 0	9 9	7.5
	6	36	7.8	9 0	5 6
	8½	36	7 8	7.5	8 3
	14	36	6 8	5 7	8 9
	24	37	6 6	5 4	8 6
10	0	36	11 7	10 4	13 9
	1½	36	15 7	16.3	14 7
	3½	36	19 9	24 1	12.5
	6	36	18 4	22 2	11 7
	7½	36	17.5	19 9	13.3
2	0	36	18 0	17 6	18 6
	3	36	19 1	21 0	15 8
	6	36	17.8	18 6	16 4

Figure 3 demonstrates that (1) a general correlation exists between the ascorbic acid concentrations of serum and cells, and that (11) the latter are consistently higher These experiments, too small in number to justify statistical treatment, also indicate that the line going through the averages is not a straight one, at increasing ascorbic acid levels, a trend towards a rise in serum concentrations, approaching equilibrium with those in the cells, is observed The distribution of ascorbic acid does not seem to depend on the cell volumes

After ingestion of ascorbic acid the concentration in serum rises above that in cells Apparently ascorbic acid gains access to the cells from serum only at a slow rate (Table II)

II Relationship between the concentration of ascorbic acid in whole blood and serum and its elimination in the urine

When following a test dose of ascorbic acid, taken by saturated subjects, the urinary elimination and the concentrations in whole blood and in serum are observed, the highest rate of excretion appears to coincide with the highest concentration in whole blood and cells and occurs approximately 1 to 2 hours after the concentration in the serum has reached its peak Figure 4 presents the data from two such experiments which are typical for all seven conducted Assuming a normal (inulin) clearance of 140 cc. per minute (27) for the subject on whom the experiment was

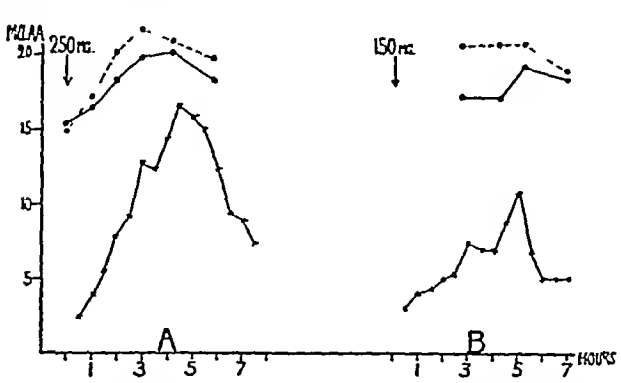


FIG 4 RELATION OF ASCORBIC ACID IN WHOLE BLOOD AND IN SERUM TO ITS URINARY EXCRETION

Arrows indicate the time of administration of ascorbic acid. x———x represents mgm. of ascorbic acid in urine, o———o and o— — —o mgm. of ascorbic acid per liter of whole blood and serum respectively

carried out, we have calculated the amounts of ascorbic acid reabsorbed per minute (Table III) The calculated concentrations of ascorbic acid per liter of cells are also presented

TABLE III

The mode of excretion of administered ascorbic acid

Time after intake	Ascorbic acid in plasma	Clearance (inulin)	Ascorbic acid filtered in glomeruli	Ascorbic acid excreted in urine	Ascorbic acid re absorbed	Ascorbic acid in blood cells
hours	*mgm per liter*	*liter per minute*	*mgm per minute*	*mgm per minute*	*mgm per minute*	*mgm per liter*
a)						
1	16 5	0 140	2 30	0 10	2 20	13 8
1½	18 0	0 140	2 52	0 15	2 37	14 1
2	19 5	0 140	2 73	0 23	2 50	14 1
2½	20 3	0 140	2 84	0 28	2 56	14 9
3	21 0	0 140	2 94	0 40	2 54	15 7
3½	20 5	0 140	2 87	0 40	2 47	16 5
4	20 2	0 140	2 83	0 46	2 37	17 8
4½	19 8	0 140	2 77	0 53	2 24	17 6
5	19 5	0 140	2 73	0 50	2 23	16 8
5½	19 0	0 140	2 66	0 45	2 21	16 2
b)						
3	20 0	0 140	2 80	0 20	2 60	10 5
3½	20 0	0 140	2 80	0 20	2 60	10 5
4	20 0	0 140	2 80	0 23	2 57	10 5
4½	20 0	0 140	2 80	0 27	2 53	13 5
5	20 0	0 140	2 80	0 30	2 50	15 9
5½	19 5	0 140	2 73	0 21	2.52	16 5
6	19 0	0 140	2 66	0 16	2 50	16 2
6½	18 5	0 140	2 59	0 13	2 46	17 1

a) from Figure 4 A
b) from Figure 4 B

These calculations show a steady increase in ascorbic acid reabsorbed per minute until 4 to 5 hours have passed, when, coincident with a further increase in urinary excretion, tubular reabsorption diminishes significantly In 5 other similar experiments in which sufficient data are available for the calculations, the same phenomena are observed

Under suitable conditions, after a saturated subject has taken a single dose of about 2 mgm of ascorbic acid per kgm of body weight *per os*, the curve of excretion in the urine may assume a biphasic form Figure 5, Number I, is typical of 7 experiments, the first peak, about 3 hours from the start of the experiment, coincides with the highest serum concentration, the second, approximately 3 hours later, marks the point when the concentration in whole blood has reached a maximum, and occurs when the concentration in serum is decreasing (Figure 4A) or constant (Figure 4 B)

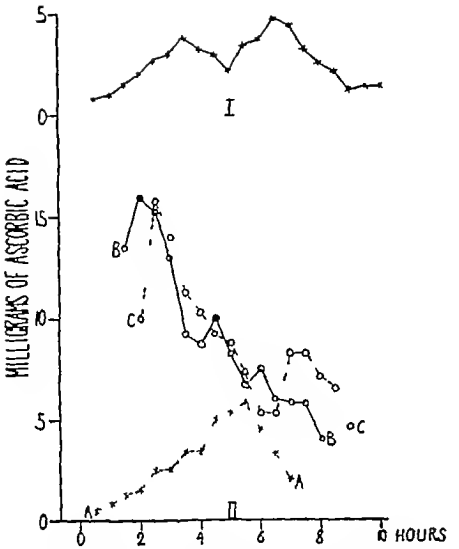


FIG. 5 CONDITIONS INFLUENCING THE MODE OF EXCRETION OF ASCORBIC ACID

In all the experiments 2 mgm per kgm. of body weight of ascorbic acid were taken at 0 hours. I After breakfast with coffee II A After 500 cc. of milk, II B and II C in the postabsorptive condition

As stated before, this biphasic excretion in urine can be observed under very special conditions only Not only must the subject be saturated and the dose carefully chosen, but the time intervening between the dose and the preceding feeding must be controlled In the 2 subjects studied, the biphasic excretion occurred consistently when the ascorbic acid was given one-half to one hour after a breakfast of cereal, buttered bread, and sweetened coffee. When the ascorbic acid was taken while the subject was in the postabsorptive state, the first peak appeared earlier and was more pronounced, while the second peak was lower (Figure 5 C) or absent (Figure 5 B), presumably owing to accelerated absorption. When ascorbic acid was taken one hour after 500 cc. of cold milk, a single peak was noted 5½ hours later (Figure 5 A), probably because absorption was so slow that a distinct peak in the serum concentration did not occur From all these experiments it is evident that absorption of ascorbic acid begins within an hour and continues for at least 4½ to 5 hours following ingestion.

In an unsaturated subject, the avidity of depleted tissues for vitamin C is such that after administration of as much as 750 mgm of ascorbic acid in a single dose the initial low concentrations in whole blood (4 8 mgm. per liter) and serum (3 0 mgm per liter) never rose above the threshold levels, consequently, the urinary excretion remained quite unaffected

The transient rise of the serum concentration above that in whole blood, however, occurs also in unsaturated subjects, if large doses are given.

III Uptoke of ascorbic acid by blood cells in vitro

The data from *in vivo* experiments already presented indicate that ascorbic acid is not distributed in the blood immediately, but that it permeates the cells slowly

From the results presented in Table IV, Numbers 1 and 2, typical of 6 such experiments, it follows that *in vitro* also ascorbic acid is taken up by blood cells but slowly Essentially the same changes are observed when ascorbic acid is not

TABLE IV

Distribution of ascorbic acid added to defibrinated blood in vitro

Ascorbic acid added	Time after addition	Cell volume	Ascorbic acid		
			Whole blood	Serum	Cells
mgm per liter	*hours*	*per cent*	*mgm per liter*	*mgm per liter*	*mgm per liter*
1 5.3	0	45	9 4	5.5	14 2
	½	45	14 1	16 4	11.3
	1	45	14.5	15.5	13.3
	2	45	14.5	15 0	13 8
	3½	45	14.5	13.5	15 8
2 6 2	0	40	9 9	7 0	14.3
	½	40	15 9	15.8	16 0
	2	40	15.5	15.8	15 0
	3	40	15 9	14 9	17.5
	4	40	15.5	13 9	18 0
3 *					
a	0	47	20 0	19 0	21 1
	4½	47	19.5	17 0	22.3
b	0	48	18 0	18 0	18.0
	4½	48	18.5	15.5	21 7

* Blood taken 1½ hours (*a*) and 5 hours (*b*) after intake of one gram of ascorbic acid

added to the shed blood *in vitro*, but is taken by the donor of the blood prior to the venipuncture (Table IV, Number 3)

DISCUSSION

I Distribution of ascorbic acid in blood

The distribution of ascorbic acid between cells and serum of fasting blood in our experiments is in essential agreement with other observations (5, 28, 29), the concentration of ascorbic acid in cells is larger than in serum or plasma when calculated for equal volumes The observations of Pijoan and Eddy (30) were taken on cells that had been washed with saline three or four times They noted higher values in serum than in the washed cells but failed to recognize the fact that in the washing of the cells ascorbic acid is withdrawn from the cell itself (31), yielding values of lower magnitude in the assay of this organic acid in blood corpuscles This view has recently been confirmed by personal communication with these investigators Confirming the results of earlier studies (5), it has been observed that the amounts of ascorbic acid in cells are not consistently proportional to those in serum, generally, as concentrations of ascorbic acid in whole blood decrease, the ratio

$$\frac{\text{concentration of ascorbic acid in cells}}{\text{concentration of ascorbic acid in serum}},$$

always greater than 1 in fasting blood, rises (Figure 3) The distribution of ascorbic acid between serum and cells, therefore, depends to some extent on the higher or lower concentration of the vitamin in the blood The observations following ingestion of ascorbic acid, presented in Table II and Figure 4, demonstrate that the concentrations of ascorbic acid in serum and in cells fluctuate more than the concentration in whole blood The exchange between the ascorbic acid present in serum and that in cells causes considerable variations in the concentrations of ascorbic acid in either one during the postabsorptive state It is the resultant of both these factors, namely whole blood, which indicates most reliably the degree of saturation in relation to the amounts of the vitamin excreted in urine Since a certain amount of vitamin is regularly ingested with normal food, the determination of this vitamin in whole blood is to be recommended also in subjects not fully saturated, since following ingestion of amounts contained in the meals, similar although less pronounced fluctuations in serum and cell concentrations occur Evidently for this reason, Wright and MacLenathen (32) withhold any vitamin C for 2 days prior to the determination of ascorbic acid in serum Furthermore, striking exceptions from the general, but not obligatory, correlation between the ascorbic acid concentrations in whole blood and in serum also support the contention that ascorbic acid should be determined in whole blood rather than in serum That the whole blood content actually indicates the degree of saturation or depletion has been demonstrated by evaluating the doses needed for saturation in individuals with different whole blood levels (Figure 2) Similar observations have been published recently by Neuweiler (8)

II The influence of ascorbic acid concentration in serum, whole blood, and cells on the rate of urinary elimination

From the data presented in Figure 4 it is evident that the rate of urinary elimination of ascorbic acid, following intake of a moderate test dose by a saturated subject, does not depend solely on the concentration of the vitamin in serum (i), but is influenced by some other factor (ii) also (i) The rate of urinary elimination of ascorbic acid rises together with, but more steeply than the concentration of ascorbic acid in serum and does not fall as soon as the concentration in the serum decreases (ii) The peak of urinary excretion of ascorbic acid coincides invariably with the peak of the concentration in whole blood

Ralli *et al* (33) showed that at very high levels of plasma ascorbic acid following intravenous injection, this vitamin is probably completely filtered in the glomeruli[3] and a maximal tubular reabsorptive capacity of 2 4 to 2 8 mgm per minute is possible At lower serum concentrations, following intake of ascorbic acid by mouth, similar rates of reabsorption were observed (Table III) From the data presented in Table III, it follows

[3] In the kidney of the frog, Leblond (34) could exclude the possibility of tubular secretion of ascorbic acid and demonstrate that urinary elimination of this vitamin is effected entirely by glomerular filtration

that two essentially different phases of urinary excretion of ascorbic acid can be distinguished Shortly after intake of ascorbic acid at rising serum concentrations, tubular reabsorption does not increase as rapidly as glomerular filtration Consequently, the amounts both reabsorbed and excreted rise. The largest excretion of ascorbic acid in urine, however, occurs in a second phase during which the serum concentrations remain constant or decline. Increased glomerular filtration can, therefore, be excluded as a cause for the increasing excretion during this period Table III shows that this further rise in the excretion of the vitamin, in spite of constant or diminishing amounts filtered in the glomeruli occurs simultaneously with a decrease in reabsorption It is during this period that the ascorbic acid concentration in the blood corpuscles reaches a peak. It might be conceived that the reaction of the cells of the renal tubules toward ascorbic acid is similar to that of the blood cells The diminished rate of reabsorption may be referable to the increasing concentration of ascorbic acid in the renal cells This increase in the ascorbic acid concentration may take considerable time as does the entrance of ascorbic acid into blood corpuscles It is reasonable to believe that the fall in power of reabsorption, related to augmented concentrations of ascorbic acid in the kidney cells, accounts for the excretory peaks that have been found to coincide with the highest concentration of ascorbic acid in blood cells *at high serum levels* Since high concentrations of ascorbic acid in red cells have been observed also at lower serum levels (Figure 3), *raised concentrations in both cells and serum* seem to be the phenomena in blood which really *characterize saturation* in the strictest sense, conditions under which maximal amounts of ascorbic acid are stored in the organism and any further intake is rapidly eliminated by the kidney No evidence is available concerning the magnitude of the amounts of ascorbic acid in the kidney under the conditions of our experiments The ascorbic acid content of the kidney is probably the more important factor for the excretion of vitamin C under normal circumstances, with concentrations of the vitamin near to the saturation level, the ascorbic acid concentration in serum assumes increasing importance for the urinary elimination when it is increased suddenly to a considerable extent by parenteral administration or by massive doses *per os*, especially during the postabsorptive state. Actually, the urinary excretion at a given moment is owing probably to the combined influences of both these factors At present, the interpretation outlined above cannot be substantiated by further experimental evidence

III Uptake of ascorbic acid by blood cells in vitro

Borsook *et al* (22) present data which they interpret as indicating impermeability of erythrocytes to ascorbic acid added to blood *in vitro*, during one hour Their data, however, agree closely with ours, after one hour the cells take up an insignificant or hardly demonstrable amount of the substance When the observations are extended to four hours, however, it becomes evident that ascorbic acid slowly penetrates the cells The experiments were not complicated by hemolysis

No explanation is offered for the transient fall in the concentration of ascorbic acid in cells noted both *in vitro* and *in vivo* when the concentration in serum is increased suddenly This phenomenon is most marked when the initial levels are high The changes are beyond the limits of error of the method, which is not more than 10 per cent, and will be the subject of further investigation

This discussion and the conclusions drawn from the experiments presented assume that the method applied is specific for ascorbic acid, a supposition supported by experimental evidence (7, 19, 20, 35) Mirsky *et al* (36), who found that whole blood values did not agree with the presumable state of saturation, failed to treat their filtrates with nitrogen long enough to completely remove H_2S, which interferes with the titration

CONCLUSIONS AND SUMMARY

In fasting blood, a general correlation exists between the ascorbic acid concentrations in cells and in serum, the concentrations in cells consistently exceed those in serum Concentrations greater in serum than in cells are observed transiently following absorption of ascorbic acid, when

the exchange between vitamin C in serum and cells causes fluctuations in their concentrations These fluctuations are more marked in both cells and serum than in their resultant, whole blood The whole blood concentration of ascorbic acid appears to correspond almost linearly to the degree of saturation of experimental subjects, it also closely indicates the different phases of complete saturation produced by test doses of ascorbic acid, as measured by the amounts excreted in urine Serum and whole blood concentrations are only roughly correlated Therefore, the determination of ascorbic acid in whole blood is to be preferred for practical purposes

The urinary elimination of large amounts of ascorbic acid following intake of a test dose by saturated subjects, depends on its concentration in the serum and on the amounts filtered therefrom in the glomeruli, and the rate of tubular reabsorption The relations between the curve of excretion and the concentrations in cells and serum suggest that the rate of reabsorption by the tubule cells may depend upon the concentration of ascorbic acid in these cells

Ascorbic acid is taken up from the plasma by red cells both *in vivo* and *in vitro* at a slow rate

BIBLIOGRAPHY

1 Emmerie, A, and van Eekelen, M, The chemical determination of vitamin C with removal of interfering reducing and coloured substances Biochem. J, 1934, 28, 1153

2 van Eekelen, M, On the amount of ascorbic acid in blood and urine. The daily human requirements for ascorbic acid Biochem. J, 1936, 30, 2291

3 van Eekelen, M, Emmerie, A, and Wolff, L K., Ueber die Diagnostik der Hypovitaminosen A und C durch die Bestimmung dieser Vitamine im Blut. Ztschr f Vitaminforsch., 1937, 6, 151

4 Deggeller, O, Onderzoekingen over het gehalte aan vitamine C van het bloed. Thesis Utrecht, 1936, Drukkerij Fa Schotanus and Jens, Utrecht.

5 Heinemann, M, On the partition of ascorbic acid between plasma and erythrocytes Acta brev Neerland, 1936, 6, 139

6 Heinemann, M I, On the relation between diet and urinary output of thiosulphate (and ascorbic acid) II Human requirements for vitamin C Biochem J, 1936, 30, 2299

7 Ferrari, R., and Buogo, G, Simplifications in the determination of ascorbic acid Arch. di fisiol, 1935, 35, 125 (cited from Nutrition Abstr and Rev 1937, 6, 649)

8 Neuweiler, W, Ueber den Nachweis des Vitamin-C Sättigungsgrades des Organismus Ztschr f Vitaminforsch, 1938, 7, 128

9 Gabbe, E, Bestimmung von Vitamin C im Blutserum. Klin. Wchnschr, 1934, 13, 1389

10 Farmer, C J, and Abt, A F, Ascorbic acid content of blood. Proc. Soc. Exper Biol and Med., 1935, 32, 1625

11 Greenberg, L D, Rinehart, J F, and Phatak, N M, Studies on reduced ascorbic acid content of the blood plasma. Proc. Soc. Exper Biol and Med., 1936, 35, 135

12 Taylor, F H L, Chase, D, and Faulkner, J M., The estimation of reduced ascorbic acid in blood serum and plasma. Biochem. J, 1936, 30, 1119

13 Pijoan, M, Townsend, S R., and Wilson, A, Determination of reduced ascorbic acid in blood Proc. Soc. Exper Biol and Med, 1936, 35, 224

14 Pijoan, M, and Klemperer, F, Determination of blood ascorbic acid. J Clin. Invest, 1937, 16, 443

15 Mindlin, R. L, and Butler, A M, The determination of ascorbic acid in plasma, a macromethod and micromethod J Biol Chem, 1938, 122, 673

16 Portnoy, B, and Wilkinson, J F, Vitamin C deficiency in peptic ulceration and haematemesis Brit M J, 1938, 1, 554
Intradermal test for vitamin C deficiency Ibid, 328

17 Lund, H, and Lieck, H, A test for ascorbic acid in serum by means of a methylene blue reaction Skandinav Arch f Physiol, 1936, 74, 269
Quantitative Bestimmung von Ascorbinsäure im Blutserum Klin Wchnschr, 1937, 16, 555

18 Elmby, A, and With, T K., Methode zur Bestimmung der Serumascorbinsäure mit Capillarblut. Die Methylenblaumethode (Lund und Lieck) als "Mikromethode." Klin Wchnschr, 1937, 16, 746

19 van Eekelen, M, and Emmerie, A, Some critical remarks on the determination of ascorbic acid Biochem J., 1936, 30, 25

20 Emmerie, A, and van Eekelen, M, The determination of ascorbic acid in blood Biochem J, 1937, 31, 2125

21 Kelhe, A E, and Zilva, S S, The catalytic oxidation of ascorbic acid. Biochem J, 1935, 29, 1028

22 Borsook, H, Davenport, H W, Jeffreys, C E. P, and Warner, R. C, The oxidation of ascorbic acid and its reduction *in vitro* and *in vivo* J Biol Chem, 1937, 117, 237

23 van Eekelen, M, and Heinemann, M, Critical remarks on the determination of urinary excretion of ascorbic acid. J Clin. Invest., 1938, 17, 293

24 Scarborough, H, and Stewart, C P, Observations on the excretion of ascorbic acid in urine. Biochem J, 1937, 31, 2232.

25 Eisenman, A. J., Mackenzie, L. B, and Peters, J P., Protein and water of serum and cells of human blood, with a note on the measurement of red blood cell volumes J Biol Chem., 1936, 116, 33

26. Heinemann, M., Requirements for vitamin C in man J Clin. Invest., 1938 17, 671
27 Smith, H W., Goldring W., and Chasis, H., The measurement of the tubular excretory mass, effective blood flow and filtration rate in the normal human kidney J Clin. Invest., 1938, 17, 263
28. Schneider E. and Widmann E., Blutserum und Bluteiweisskörper und Vitamin C. Klin. Wchnschr., 1935, 14, 1454
29 Stephens, D J., and Hawley, E. E., The partition of reduced ascorbic acid in blood. J Biol. Chem., 1936 115, 653.
30 Pijoan, M., and Eddy E., Ascorbic acid content of red cells and plasma. J Lab and Clin. Med., 1937 22, 1227
31 Heinemann, M., and Heinemann, G., Unpublished studies.
32. Wright, L S and MacLenathen, E., Vitamin C saturation—kidney retention after an intravenous test dose of ascorbic acid. Proc. Soc. Exper Biol and Med., 1938 38 55
33 Ralli E. P., Friedman, G J and Rubin, S H., The mechanism of vitamin C excretion in man studied by simultaneous vitamin C and inulin clearances. J Clin. Invest. (Proc.) 1938, 17, 504
34 Leblond C. P., Mécanisme de l élimination rénale de la vitamine C. Compt. rend. Soc. de Biol., 1938, 127 208.
35 Manceau, P, Policard, A., and Ferrand M., Remarques sur le dosage chimique de l'acide ascorbique. II Étude critique des procédés de purification. Application au dosage dans l urine. Bull. Soc. chim. biol., 1936 18, 1623
36 Mirsky I A., Swadesh, S., and Soskin, S., Total ascorbic acid content of human blood. Proc. Soc. Exper Biol. and Med., 1935 32, 1130

THE EXCRETION OF PORPHYRINS IN CONGENITAL PORPHYRIA

By KONRAD DOBRINER, W H STRAIN H GUILD AND S A. LOCALIO

(From the Department of Medicine The University of Rochester School of Medicine and Dentistry Rochester New York The Harriet Lane Home Johns Hopkins Hospital Baltimore and The Hospital of the Rockefeller Institute New York City)

(Received for publication July 22, 1938

In 1915 Hans Fischer identified the kinds and types of porphyrins excreted in congenital porphyria (1, 2, 3), and since that time 8 cases of this rare disease have been studied by qualitative, chemical methods (4 to 11) No quantitative studies of the total excretion of porphyrins in this condition have been made, however, and although reports of the therapeutic effect of liver extract on acute porphyria are recorded in the literature (12, 13, 14), complete detailed observations have not been published In this communication qualitative studies of the types and kinds of porphyrins excreted in 3 cases of congenital porphyria are recorded, together with quantitative studies of the coproporphyrin excretion and the effect of liver extract therapy on that excretion in 2 of the cases

The methods used for the qualitative and quantitative determinations of the urinary and fecal porphyrins, except uroporphyrin, are those previously reported (15, 16) Uroporphyrin was isolated by the method of Fischer and Duesberg (11), but because of the lack of a suitable method it was not measured quantitatively In all instances a thorough search for isomeric and hitherto undescribed porphyrins was made.

The clinical material studied was as follows Case I (Rochester) (20) A clinical report has been published (29) Case II (Baltimore), Hospital record number 98673, Harriet Lane Home, Johns Hopkins Hospital Case III (San Francisco) A preliminary report concerning the photosensitivity in this child has already been published by Blum and Hardgrave (17)

RESULTS

Qualitative

In all 3 cases both coproporphyrin and uroporphyrin were present in large amounts in the urine. Much coproporphyrin was present in the feces, as well as relatively small amounts of protoporphyrin and deuteroporphyrin In Table I the results of the melting point determinations

TABLE I

Melting points in ° C of the porphyrin methyl esters

Case number	Urine		Feces, *Copro I*	Free coproporphyrin of natural ester
	Copro I	*Uro I*		
I (Rochester)	250	286	249	251
II (Baltimore)	251	285	250	
III (San Francisco)	249	279		

are given In Case I relatively large amounts of a natural coproporphyrin ester (15*b*) were present both in the urine and in the feces but it was not found constantly This natural ester had an HCl number of 0 3 to 0 5 per cent HCl, and was easily extracted from this HCl concentration with chloroform It could be saponified with 20 per cent NaOH After saponification the porphyrin was no longer soluble in chloroform and showed all the properties of coproporphyrin Spectroscopically it was identical with coproporphyrin Althougb the esterifying group could not be established definitely, certain qualities suggested that it was of a lipoidal nature. The Liebermann Burchard reaction was negative. After saponification the porphyrin was esterified and the methyl ester was identified by melting point determinations as coproporphyrin I (*M P* 251° C.)

Quantitative

In Cases I and II quantitative determinations of coproporphyrin excretion in the urine and feces could be made during control periods and during periods of intensive intramuscular liver extract therapy

Case I (Rochester) Figure 1 A 3-year-old, female child was admitted to the Strong Memorial

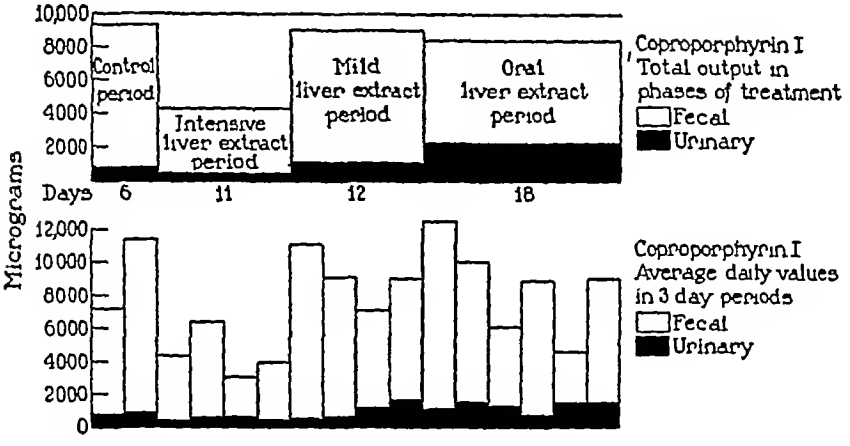


FIG 1 CASE I

Hospital, Rochester, New York, in August, 1936, and was maintained on a meat free diet during the study Quantitative studies of coproporphyrin were made both of the urine and feces on 3-day collections A control period of 6 days was followed by a period of 11 days, during which 5 cc. of concentrated liver extract (Eli Lilly and and Co) was injected intramuscularly each day In a subsequent period of 12 days, 5 cc. of liver extract was administered twice weekly, and in a final period of 18 days 10 to 30 grams of liver extract (Lilly) was administered daily by mouth

The total excretion of coproporphyrin during the control period was about 50 times the normal average for a child The average daily total output was 9380 micrograms, of which 870 micrograms were excreted in the urine Following daily injections of liver extract the total coproporphyrin output decreased to an average of 4450 micrograms a day, of which 550 micrograms were in the urine In the third period the excretion of coproporphyrin increased rapidly, approaching the levels present before treatment The total coproporphyrin output during this period averaged 9250 micrograms, of which 1060 micrograms were excreted in the urine. In a fourth period, of 18 days' duration, the patient received from 10 to 30 grams daily of liver extract by mouth, and 2 injections of intramuscular liver extract weekly The coproporphyrin output in this period averaged 8890 micrograms of which 1240 micrograms were excreted in the urine During the period of intensive liver extract therapy the uroporphyrin excretion decreased, and the natural porphyrin ester excreted during the control period could no longer be detected Clinical improvement was manifested by the disappearance of the vesicular eruption

Case II (Baltimore) Figure 2 The patient was a 4-year-old girl observed from 1936 to 1938 in the Harriet Lane Home and maintained on a constant diet The urine and feces were collected in 3-day periods and studied at the Hospital of the Rockefeller Institute In the control period of 15 days the child excreted an average of 5600 micrograms of coproporphyrin daily, of which 1535 micrograms were excreted in the urine In the treatment period of 12 days the patient received 5 cc of liver extract (Lederle) intramuscularly each day The total coproporphyrin excretion decreased to 1650 micrograms daily, of which 520 micrograms were in the urine In a second control period of 12 days the total average coproporphyrin output rose to 6030 micrograms daily, of which 1670 micrograms were in the urine

DISCUSSION

The 3 cases reported showed a mass excretion of coproporphyrin I and uroporphyrin I similar to the 6 cases of congenital porphyria previously described in the literature (1 to 8) Two cases of congenital porphyria with a mass excretion of coproporphyrin III also have been described elsewhere (9, 10, 11) Fischer and Hofmann (18) recently reported that in a restudy of the uroporphyrin fraction of the famous case, Petry, small amounts of uroporphyrin III were isolated from the large uroporphyrin I fraction Uroporphyrin I was excreted by the cases here re-

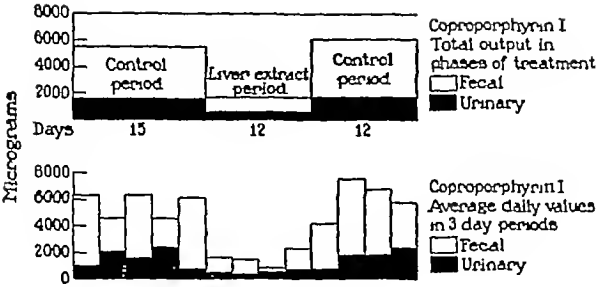


FIG. 2. CASE II

ported, but small amounts of uroporphyrin III may also be present Work on this phase of the problem is still in progress The coproporphyrin methyl ester fraction was separated by a method previously described (15, 19) and in no instance was coproporphyrin III obtained from the large amounts of coproporphyrin I present

A working hypothesis which has been outlined previously (16, 20, 21, 22, 23, 24) is derived from the *in vitro* synthesis of the porphyrins and is supported by clinical and experimental evidence. The hypothesis postulates the simultaneous construction of Types III and I porphyrins in nature Under normal conditions there appears to be a relatively constant ratio between the amounts of the 2 types formed

In congenital porphyria (20) with mass production and excretion of Type I porphyrins the normal ratio between the construction of Type III and Type I compounds is disturbed and a disproportional or disorderly type of synthesis in favor of Type I occurs In recent publications Rimington independently has come to similar conclusions (25, 26) From these studies together with those previously reported it appears that the disturbance of porphyrin metabolism which characterizes congenital porphyria is quite unlike that seen in pernicious anemia (23) in pellagra (27), or in refractory anemia (28)

SUMMARY

1 In 3 cases of congenital porphyria in children qualitative porphyrin studies revealed a mass excretion of coproporphyrin Type I and uroporphyrin Type I In 1 case of the 3 a natural coproporphyrin I ester was excreted

2 Quantitative studies in 2 of the 3 cases suggest that the porphyrin excretion in this disease is influenced by daily injections of liver extract

We are greatly indebted to Dr W S McCann, and Dr S W Clausen for their cooperation and assistance.

BIBLIOGRAPHY

1 Fischer H., Über das Uroporphyrin. I. Ztschr f physiol. Chem., 1915 95, 34
2. Fischer, H Über das Kotporphyrin. II Über das Urinporphyrin. Ztschr f physiol. Chem., 1915, 96, 148.
3 Fischer H., and Orth, H., Die Chemie des Pyrrols, 2 (Part I) Leipzig 1937
4 Fischer H., and Zerweck, W., Über den Harnfarbstoff bei normalen und pathologischen Verhältnissen und seine lichtschutzende Wirkung Zugleich einige Beitrage zur Kenntnis der Porphyrinurie. Ztschr f physiol. Chem., 1924 137, 234
5 Mackey, L. and Garrod, A. E., A further contribution to the study of congenital porphyrinuria (haematoporphyria congenita) Quart. J Med., 1926 19, 357
6 Ashby H T., Haematoporphyria congenita (congenital porphyrinuria) its association with hydroa vaccineiforme and pigmentation of the teeth Quart. J Med., 1926, 19 375
7 Jost, H., Medizinisch biologischer Abend der medizinischen Fakultät Frankfurt. a. M Klin. Wchnschr., 1927 6, 827
8. Waldenstrom, J., Studien über Porphyrie. Acta med. Scandinav., Supp. 82, 1937, 22.
9 Van den Bergh A. A. H., Regniers and Muller, Ein Fall von kongenitaler Porphyrinurie mit Koproporphyrin in Harn und Stuhl. Arch. f Verdauungskr., 1928 42, 306.
10 Fischer H., Platz, K., and Morgenroth, K., Synthese von Koproporphyrin. III und IV ein Beitrag zur Kenntnis der Porphyrie. Ztschr f physiol. Chem., 1929 182 265

minute interval, which was allowed for mixing time, 3 control periods of 10 minutes each were run with the plasma vitamin C at its normal level An intravenous injection containing from 1500 to 6000 mgm of vitamin C was then given Additional urine specimens were collected while the vitamin C concentration in the plasma was falling from this high level (Figure 1)

In this first series, the plasma concentration of the vitamin varied from 1 89 to 34 9 mgm per cent The vitamin C clearance has a low value when the plasma level is below 2 mgm per cent, and as the plasma level is raised, the clearance rises rapidly and approaches the inulin clearance as a limiting value This physiological relationship is illustrated in Figure 2, which shows the vitamin C/inulin clearance ratio in relation to the plasma level of vitamin C in 82 clearance periods in 4 subjects (This figure also includes the data reported below)

Analysis of these data suggested that the elevation of the vitamin C clearance at the higher plasma concentration was owing to the fact that the renal tubules reabsorb the vitamin up to some maximal limiting rate, after which any vitamin present in the glomerular filtrate is excreted in the urine A second series of experiments, therefore, was designed to examine this point specifically Simultaneous inulin and vitamin C clearances were determined at three steadily maintained plasma levels of vitamin C in the following manner On the day prior to the observations sufficient vitamin C was given by mouth to raise the blood level to 2 mgm per cent Inulin was administered as before and three urine specimens were collected at this plasma level Four hun-

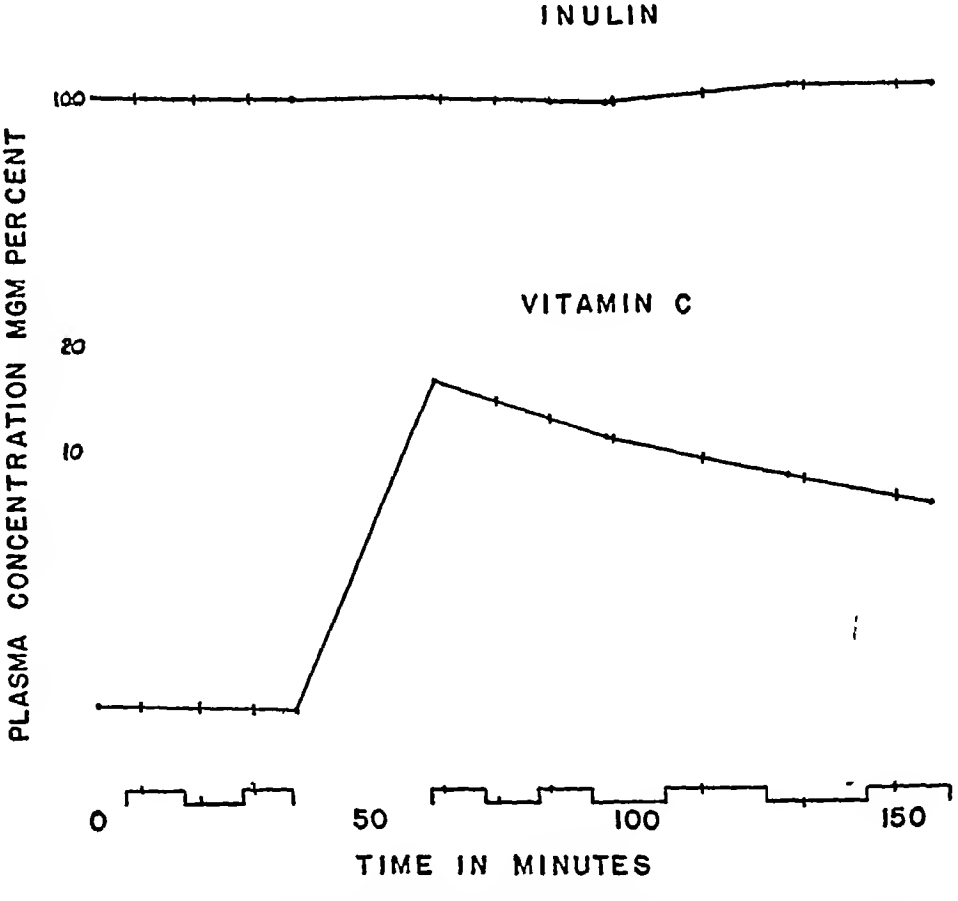


FIG 1 EXPERIMENT SHOWING VITAMIN C AND INULIN PLASMA CONCENTRATIONS

The boxes at the base refer to the urine collection periods The dots represent the plasma concentrations The bars on the plasma curves are the values used in the calculations of the clearances

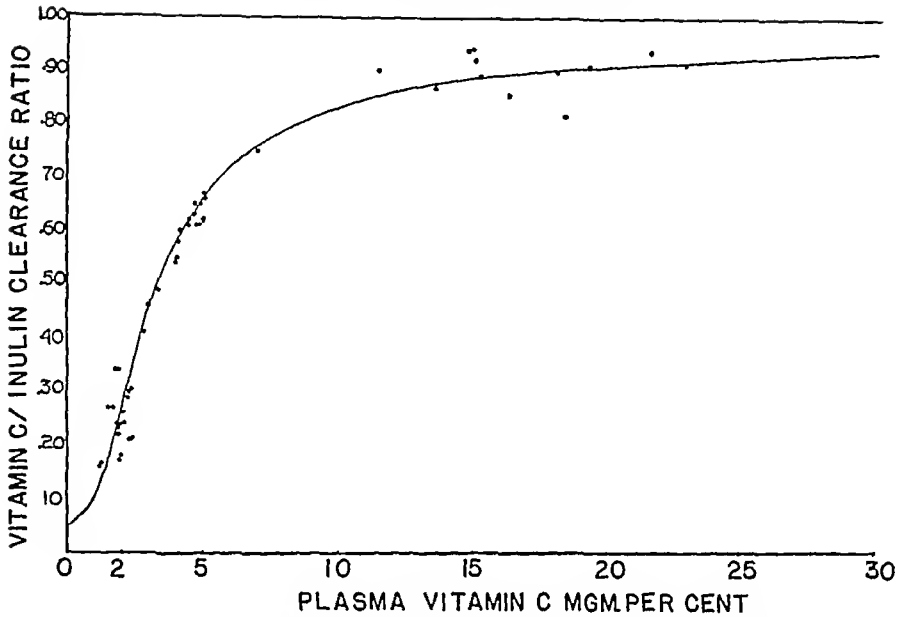


FIG. 2. VITAMIN C/INULIN CLEARANCE RATIOS PLOTTED AGAINST PLASMA CONCENTRATION OF VITAMIN C
The line is calculated from Equation 1 given in the text, and the dots represent the individual clearance ratios.

dred mgm of vitamin C were injected intravenously to raise the plasma concentration to 4 mgm per cent Following this, a second infusion of 450 cc. of 3 per cent inulin and an additional 400 mgm of vitamin C were given to maintain the plasma concentration at this level Three urine specimens were collected at this intermediate level Then another priming dose of vitamin C (2000 mgm.) was injected intravenously and the infusion changed to one containing 2000 mgm of the vitamin in 450 cc of 3 per cent inulin This served to maintain the plasma level of vitamin C at about 20 mgm per cent. Three urine specimens were collected at this high plasma level At each stage a 20-minute interval was allowed for mixing time.

The absolute amount of vitamin reabsorbed by the tubules was calculated by subtracting the amount excreted per minute from the amount filtered per minute. Assuming that the vitamin is completely filtrable from the plasma, the latter value is obtained by multiplying the inulin clearance by the plasma concentration of the vitamin The determination of the amount reabsorbed is most accurate when the plasma level of vitamin C lies between 3 and 10 mgm per cent, as at the higher plasma levels an error of 1 per cent in the determination of either the inulin or vitamin C clearance will introduce an error of 14 per cent in the calculation of the absolute amount of vitamin C reabsorbed

Table I gives the quantity of vitamin C reabsorbed by the tubules in 53 observations in 3 subjects at 3 different plasma levels, each figure being the average of 3 observations Expressing all functions in terms of 100 cc of glomerular filtrate serves to eliminate in part the variations resulting from differences in the size of the kidneys in the different subjects

These data indicate that there is in fact an absolute limitation in the capacity of the tubules to reabsorb the vitamin

TABLE I

Maximal reabsorption of vitamin C

Subject	Plasma concentration	Filtered		Excreted		Reabsorbed	
			Glomerular filtrate		Glomerular filtrate		Glomerular filtrate
	mgm per cent	*mgm per minute*	*mgm per 100 cc*	*mgm per minute*	*mgm per 100 cc*	*mgm per minute*	*mgm per 100 cc*
P B	1 98	2 56	1 98	0 47	0 36	2 09	1 62
	4 94	6 17	4 94	3 95	3 16	2 22	1 78
	21 6	27 6	21 6	26 0	20 4	1 6	1 2
P B	1 94	2 57	1 94	0 90	0 68	1 67	1 26
	15 1	20 3	15 1	18 7	13 9	1 6	1 2
H M	2 36	2 65	2 36	0 58	0 51	2 07	1 85
	5 10	5 90	5 10	3 97	3 43	1 93	1 67
	13 6	16 3	13 6	14 0	11 7	2 3	1 9
H M	2 10	2 43	2 10	0 60	0 52	1 83	1 58
	4 92	5 58	4 92	3 33	2 94	2 25	1 98
	10 6	12 7	10 6	9 9	8 2	2 8	2 4
L R	1 63	1 99	1 63	0 05	0 04	1 94	1 59
	4 14	4 95	4 14	2 88	2 40	2 07	1 74
	18 4	24 1	18 4	22 4	17 2	1 7	1 2
L R	2 30	3 05	2 30	0 92	0 69	2 13	1 61
	4 64	6 32	4 64	4 00	2 94	2 32	1 70
	18 4	25 2	18 4	20 9	15 2	4 3	3 2

circumstance that leads to the increased excretion at elevated plasma levels The agreement in the figures for the maximal rate of reabsorption is as good as may be expected in view of the possible errors in the method Because of the nature of the calculation, the data at intermediate plasma levels are more significant than those at high plasma levels

The third type of experiment was designed to determine whether vitamin C and glucose are reabsorbed by a common mechanism This inquiry was stimulated by the fact that vitamin C is closely related in chemical structure to the carbohydrates Two series of observations were conducted on one subject in the following manner In the first, the subject was given enough vitamin C the day before to raise the plasma level to 2 mgm per cent Inulin was given as before, and three 10-minute urine specimens were collected A priming dose of 25 cc. of 50 per cent glucose was then injected, and glucose was added to the inulin infusion fluid (10 cc per minute), in sufficient quantity to give a concentration of 10 per cent No additional vitamin C was given In a second series of observations, the plasma concentration of glucose was maintained at 3 levels Enough vitamin C was given the day before to raise the plasma level to 2 mgm per cent, and three clearances were determined at normal plasma glucose levels A priming dose of 50 cc of 50 per cent glucose was then given, with a sustaining infusion of 8 3 per cent glucose (10 cc per minute) Three urine specimens were collected at this intermediate glucose level, and then an additional 50 cc of 50 per cent glucose was given with a sustaining infusion of 14 8 per cent glucose (10 cc per minute) Three more urine specimens were collected at this higher plasma glucose level

The results, given in Table II, show that when the plasma glucose is raised to levels where the glucose reabsorptive mechanism is presumably saturated, *i e* above 300 mgm per cent (17), the reabsorption of vitamin C is unimpaired From this it may be inferred that the reabsorption of vitamin C and of glucose do not involve a common mechanism The data were also analyzed with a view to determining whether or not the rate of urine flow affected the excretion of vitamin C Absolutely no relationship was found between these two when the urine flow varied from 1 5 to 15 cc per minute

TABLE II

Effect of hyperglycemia on the reabsorption of vitamin C

Plasma vitamin C	Plasma glucose	Vitamin C/inulin clearance ratio	Vitamin C reabsorbed
mgm per cent	*mgm per cent*		*mgm per minute*
2 02	88	0 33	1 94
2 00	88	0 32	2 17
1 97	90	0 30	2 10
1 79	275	0 60	1 15
1 75	290	0 33	2 02
1 70	310	0 32	1 68
1 54	525	0 31	1 52
1 53	522	0 27	1 93
1 52	515	0 25	2 16
1 52	510	0 24	2 15

DISCUSSION

The validity of the calculation of the active tubular reabsorption depends on the assumption that the vitamin is completely filtrable at the glomeruli This question has not been examined experimentally because of difficulties in preventing the oxidation of the vitamin, but Leblond

(18) has reported that vitamin C is present in the capsular fluid of the frog in the same concentration as in the plasma We believe that our results warrant quantitative treatment in terms of the assumption that the vitamin is also completely filtrable in man The data indicate that vitamin C is excreted only by filtration, that it is actively reabsorbed by the renal tubules, and that the factor which limits this reabsorptive process is the existence of a maximal rate, such as has been demonstrated in the tubular reabsorption of glucose (17) and the tubular excretion of phenol red (19, 20, 8), diodrast (8), and a number of other substances (21) Taking only those observations in which the plasma vitamin C is between 2 and 5 mgm per cent, this maximal rate is essentially the same in each of these individuals (2 16 mgm per minute or 1.77 mgm per 100 cc. of glomerular filtrate)

It follows that the quantity of vitamin C excreted in the urine of a given individual will be determined by the plasma concentration of the vitamin, by the rate of glomerular filtration, and by the maximal rate of tubular reabsorption. Accepting this fact, we have attempted to describe the excretory process in a quantitative manner, as has been done by Shannon and Fisher (17) for glucose. To calculate the smooth curve in Figure 2 the rate of tubular reabsorption, T_r (mgm per minute), is calculated at various plasma concentrations of vitamin, a (mgm per cent), from Shannon and Fisher's working equation,

$$\left(a - \frac{T_r}{V}\right)\left(\frac{T_m - T_r}{T_r}\right) = K, \qquad (1)$$

where T_m is the maximal rate of tubular reabsorption, V is the rate of glomerular filtration in units of 100 cc., and K is a constant. In this equation, when V, the rate of glomerular filtration, is reduced to 100 cc. per minute, T_m has a value of 1.77 mgm per 100 cc. glomerular filtrate, and K has a value of 0 1 Using the values of T_r obtained and inserting at the selected values of a, the vitamin C/inulin clearance ratio is calculated according to the equation

$$\text{Vitamin C/inulin clearance ratio} = 1 - \frac{T_r}{a} \qquad (2)$$

The calculated ratio agrees satisfactorily with the observed data, and the fact of this agreement, especially at elevated plasma concentrations of vitamin C, supports the assumption that the vitamin is completely filtrable from the plasma.

It should be noted that according to Equation 1 the vitamin C clearance does not become zero at very low plasma levels, but ultimately falls to a minimal value which is independent of plasma level This minimal clearance is determined by the relative magnitudes of the constant K the rate of filtration, V, and the capacity of the renal tubules to reabsorb the vitamin, as revealed in T_m Probably, the theoretical minimal clearance would not be reached in any subject except in prolonged vitamin C deficiency But even in view of this theoretical prediction, it is evident that the vitamin will continue to be excreted in the urine so long as it is present in the blood, *i e*, the renal mechanism of reabsorption offers no safeguard against complete depletion of the vitamin in the body when the intake or synthesis is zero Further experiments at this very low level are being done to get satisfactory data regarding this point

Giroud and Leblond (22) studying the renal elimination of ascorbic acid by histological technique noted that when ascorbic acid was given intravenously to guinea pigs, it was found in the cells of the proximal convoluted tubules and the descending branch of Henle's loop None was observed in the ascending branch of Henle's loop, the distal convoluted tubules, or the excretory ducts Our observations prove that vitamin C is reabsorbed by the renal tubules These observations would indicate that this process is a function of the proximal portion of the renal nephron

SUMMARY

1 Simultaneous vitamin C and inulin clearances show that vitamin C is excreted by filtration and active tubular reabsorption

2 The reabsorptive mechanism for vitamin C appears to be limited by a maximal rate, so that when the vitamin is presented to the tubules by the glomerular filtrate at a rate exceeding this maximum the excess is excreted in the urine.

3 The maximal rate of reabsorption in three individuals averages 2 16 mgm per minute (or 1.77 mgm per 100 cc. of glomerular filtrate)

4 The excretion of vitamin C in a given individual will be determined by (1) the plasma level, (2) the rate of glomerular filtration, and (3) the

maximal rate of tubular reabsorption The nature of the reabsorptive process is such that at low plasma levels the vitamin C clearance reaches a minimal and constant value

5 Although in chemical structure vitamin C is related to the carbohydrates, it is not reabsorbed by the same mechanism as glucose

We are indebted to Merck & Company for the cevitamic acid used in this work.

BIBLIOGRAPHY

1 Harris, L J, Ray, S N, and Ward, A, The excretion of vitamin C in human urine and its dependence on the dietary intake. Biochem J, 1933, 27, 2011

2 Youmans, J B, Corlette, M B, Akeroyd, J H, and Frank, H, Studies of vitamin C excretion and saturation Am J M Sc., 1936, 191, 319

3 Hawley, E E, Stephens, D J, and Anderson, G, The excretion of vitamin C in normal individuals following a comparable quantitative administration in the form of orange juice, cevitamic acid by mouth and cevitamic acid intravenously J Nutrition, 1936, 11, 135

4 Wright, I S, Lilienfeld, A, and MacLenathen, E, Determination of vitamin C saturation A 5 hour test after an intravenous test dose. Arch. Int. Med, 1937, 60, 264

5 Wortis, H, Liebmann, J, and Wortis, E, Vitamin C in the blood, spinal fluid and urine. J A M A, 1938, 110, 1896

6 Faulkner, J M, and Taylor, F H L, Observations on the renal threshold for ascorbic acid in man J Clin. Invest., 1938, 17, 69

7 Ralli, E. P., and Friedman, G J, The response to the feeding of cevitamic acid in normal and deficient subjects as measured by a vitamin C excretory test. Ann Int. Med., 1938, 11, 1996

8 Smith, H W, Goldring, W, and Chasis, H, The measurement of the tubular excretory mass, effective blood flow and filtration rate in the normal human kidney J Clin Invest., 1938, 17, 263

9 Farmer, C. J, and Abt, A F, Ascorbic acid content of blood Proc. Soc. Exper Biol and Med., 1935, 32, 1625

10 Birch, T W, Harris, L J, and Ray, S N, A microchemical method for determining the hexuronic acid (vitamin C) content of foodstuffs, etc. Biochem J, 1933, 27, 590

11 Emmerie, A., and van Eekelen, M, The chemical determination of vitamin C with removal of interfering reducing and coloured substances Biochem J, 1934, 28, 1153

12 van Eekelen, M, and Heinemann, M, Critical remarks on the determination of urinary excretion of ascorbic acid. J Clin Invest., 1938, 17, 293

13 Harris, L J, and Ray, S N, Diagnosis of vitamin C subnutrition by urine analysis with a note on the antiscorbutic value of human milk. Lancet, 1935, 1, 71

14 Harris, L. J, Chemical test for vitamin C and the reducing substances present in tumor and other tissues Nature, 1932, 132, 27

15 Friedman, G J., Rubin, S H, and Kees, W, Effect of addition of KCN to whole blood on indophenol-reducing power of plasma Proc. Soc. Exper Biol and Med, 1938, 38, 358

16 Farmer, C J, and Abt, A F, Invalidation of plasma ascorbic acid values by use of potassium cyanide. Proc. Soc Exper Biol and Med., 1938, 38, 399

17 Shannon, J A, and Fisher, S, The renal tubular reabsorption of glucose in the normal dog Am J Physiol, 1938, 122, 765

18 Leblond, C P, Méchanisme de l'élimination rénale de la vitamine C Compt. rend. Soc. de biol, 1938, 127, 208

19 Shannon, J A, The excretion of phenol red by the dog Am J Physiol, 1935, 113, 602.

20 Pitts, R. F, The excretion of phenol red by the chicken. J Cell. and Comp Physiol, 1938, 11, 99

21 Shannon, J A, Renal tubular excretion. Physiol. Rev (In press)

22 Giroud, A, and Leblond, C P, Histological study of renal elimination of ascorbic acid Anat. Rec., 1937, 68, 113

STUDIES IN TEMPERATURE SENSATION IV THE STIMULATION OF COLD SENSATION BY RADIATION

By JAMES D HARDY and THEODORE W OPPEL

(From the Russell Sage Institute of Pathology in affiliation with the New York Hospital and Department of Medicine Cornell University Medical College New York City)

(Received for publication August 10 1938)

The first papers of this series (1, 2, 3) reported methods of using radiant energy as a purely thermal stimulus and demonstrated that the sensory effects of radiation depend upon the temperature changes produced in the skin by the absorption of various spectral bands The threshold stimulus which activates a warmth sensitive end organ was measured and was differentiated from the sensory threshold of warmth The sensory threshold was shown to vary when different parts of the body surface were exposed to the stimulus, and this variation depended largely upon the end organ population of the part and upon the ability of the central nervous system to combine or summate discharges resulting from the activation of numerous end organs On certain parts of the body surface the end organ population appeared to be uniform, and here the relationship between the strength of the threshold stimulus and the size of the stimulated area followed a mathematical formula. This was assumed to be the formula for the summation of end organ responses from that region of the body surface.

The psychological aspects of cold sensation have been studied extensively by Goldscheider (4), Levine and Dallenbach (5), and others, and the physiology by Bazett and McGlone (6) and more recently by Nafe and Wagoner (7) The importance of temperature sense in the regulation of body heat loss has been recently emphasized by Herrington, Winslow, and Gagge (8), by Jung, Doupe, and Carmichael (9), and by Du Bois (10) The past studies of cold sensation have not been concerned essentially with the relation of cold sense to regulation of body temperature, and it was hoped that with the radiation method more could be learned about how the human body recognizes the temperature of objects in its environment and that any similarities or differences associated with the mechanism of the perception of warmth and cold would be found As all objects of the temperature of the skin surface or colder radiate in the spectral region longer than 4 μ, and inasmuch as the skin has the same optical properties throughout this entire spectral range, we are not concerned with the effects of different wave lengths in the present study The problem is limited, therefore, to a determination of the threshold stimuli for cold and to a comparison of the summation of responses from cold end organs with the summation of responses from heat end organs

EXPERIMENTAL

In the present experiments the technique previously used has been altered to apply to an investigation of cold sensation. The sense of cold was stimulated by exposing a portion of the body surface to a block of solid CO_2. The radiant exchange between the skin and the CO_2 is conveniently termed "cold" radiation.[1] In these experiments the skin which has become adapted to the general environment, is suddenly exposed to an object much below room temperature so that the heat loss from the skin by radiation is greatly increased. This *increase* in heat loss is the quantity which is measured as cold" radiation and is actually the heat loss of the environment by radiation to the cold object. Thus objects below the general environmental temperature will be sources of cold radiation and those above will be sources of heat radiation.

Measurement of the minimum stimulating amount of cold radiation

The apparatus used to measure the minimum stimulating amount of cold radiation was essentially the same as that used for determining the minimum stimulating amount of heat radiation and is described in detail in the first paper of this series (1) In place of the incandescent lamp or electric stove used as sources of radiant heat (S in Figure 1 of Paper 1) a block of CO_2 snow, 20 × 20 cm. was used as a source of cold radiation A silver lined truncated cone (30 cm. long 22.3 cm. in diameter at its base and 7.7 cm. in diameter at its truncated

[1] As the term "cold" radiation already appears in standard textbooks of physics (See Edwin Edser, Heat for Advanced Students McMillan and Co., 1929) and is being used by students and engineers interested in air conditioning acceptance of the term definition appear desirable.

end) served to concentrate the rays in place of the lens which focused the heat rays in some of the former experiments No filters were used. The strength of the radiation was altered by moving the block of snow towards or away from the subject. A cardboard shutter was held between the CO_2 block and the cone, and after the subject had become accustomed to the sensations of the apparatus and the room, the shutter was removed and a stimulating amount of radiation allowed to fall on the subject's forehead for 1 second This length of time was used for cold stimulation rather than the 3 seconds used for warm stimulation because the cold was perceived much quicker than the warmth Repeated tests were made until the smallest amount of cold radiation which the subject recognized accurately in this time was found, the strength of the radiation was determined with a small radiometer in the same manner that the warm radiation stimulus was measured

Circular holes cut in pieces of cardboard limited the size of the skin area irradiated, and threshold stimuli were determined for 8 areas ranging from 3 46 sq cm. to 1986 sq cm The same technique was used to measure reflection of cold radiation that had been used to measure infra-red reflection Cold reflection was found to be too small (less than 2 per cent) to effect the present results

Measurements of the thermal changes resulting from cold radiation

Skin temperature changes resulting from cold radiation were measured by the same technique with which the changes resulting from warm radiation were measured, and that method is described in detail in the second paper of this series (2) The apparatus is shown in Figure 1 of that paper When the silver lined cone was used to augment the cold radiation, it was mounted on the same axis as the skin temperature measuring radiometer in such a way that it reflected the radiation onto the skin surface while the radiometer was before the constant temperature reference body Swinging the radiometer into position for measuring skin temperature automatically moved the cone to one side. Curves of the heating of the skin after cold radiation (corresponding to cooling after heat radiation) were formed and from them constant time charts were constructed They are shown in Figure 1 The details of their formation and their interpretation are the same as for heat radiation and are described elsewhere (2) The lines on this chart show the average cooling of the skin surface by various strengths of cold radiation applied for 15, 30, 45, and 60-second periods Skin temperature changes reported in this paper are all derived from these charts

Early in the present investigation it became apparent that there was a marked difference between the thermal and stimulating effects of the same quantities of radiant heat and cold Because of the limited available intensity of cold radiation it was necessary to measure even smaller skin temperature changes than had to be measured in the experiments with warmth Therefore, a number of tests were made to determine the accuracy of the methods

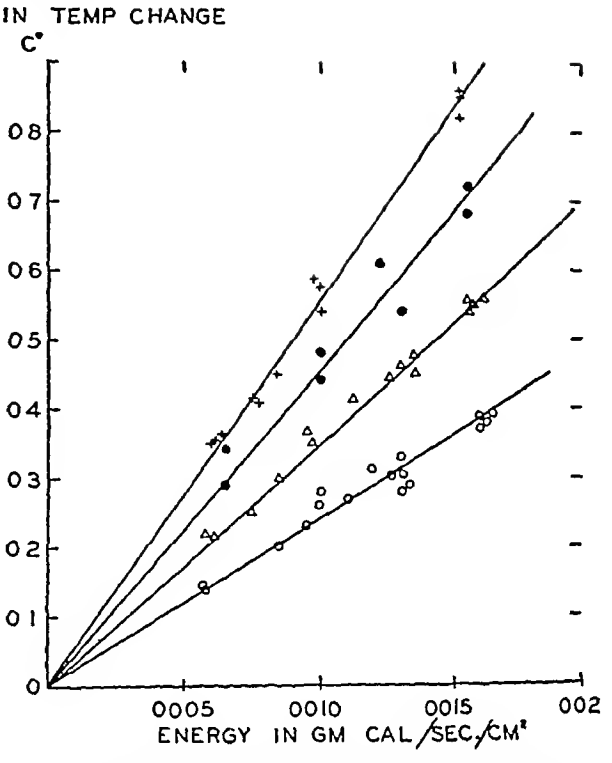


FIG 1 CONSTANT TIME CURVES FOR COLD RADIATION +, 60 seconds exposure, ●, 45 seconds exposure, Δ, 30 seconds exposure, ○, 15 seconds exposure.

Calibration of skin temperature radiometer

As the method of determining the amount of the change in skin temperature produced by a given exposure to the CO_2 involved continuous observation of the skin temperature for some minutes after the exposure, the following experiment was devised to test the procedure. Two Leslie cubes were mounted so that the radiometer could swing readily from one to the other, the thermopile of the radiometer facing directly into the cone of each cube. The water in each cube was stirred constantly by a motor and maintained at constant (± 0 01° C) temperatures by thermostatically controlled heaters The temperature of the water was measured by U S Bureau of Standards certified thermometers, accurate to 0 001° C.

One cube served as a constant temperature reference body at 33 70° C and the radiometer standardized against it The radiometer was then swung over to the other cube, which was maintained at 34 55° C. for 3 minutes, when the heater was turned off and the cube allowed to cool At intervals of one minute the cube thermometer was read and simultaneously the difference between the temperatures of the two cubes was measured with the radiometer The results are shown in Figure 2 Ther-

mometer readings are plotted as solid dots. Radiometer readings as circles. As there was less than 0 005 C. difference between the temperature readings by these two methods it can be assumed that the radiometer will accurately follow the skin temperature changes for some minutes.

Measurement of temperature changes produced by heat and cold radiation on a blackened metal plate

To make sure that the observed difference in the thermal properties of heat and cold radiation for the skin was not owing to the experimental procedure, the heating and cooling of a thin blackened, copper plate was observed. One Leslie cube served as a constant temperature reference body and a circular copper plate was mounted 3 mm in front of the cone of a second Leslie cube, which was also kept at a constant temperature. The front surface of the plate was blackened and a thermocouple was soldered in the middle of it so that its temperature could be continually recorded. After the plate had come to thermal equilibrium it was irradiated with 0 00105 gm./cal./cm.²/sec. of heat for periods of 30 and 60 seconds and the subsequent cooling after ir radiation was measured by the radiometer The procedure was repeated, using the same number of calories of cold radiation for the same time periods. The results are shown in Figure 3 Almost identical temperature changes were found after warm and cold irradiation, and there was close agreement between the temperature measured with the radiometer and by the thermocouple.

From the above control experiments we feel that the radiometric measurement of surface temperature by our method is accurate to ± 0 005° C.

RESULTS

The minimum perceptible cold radiation was measured for several areas on six subjects, all of whom were found to have approximately the same sensory thresholds On two of these subjects a detailed study of the sensory threshold for cold radiation was made by exposing 8 different sized areas to the radiation stimulus The 6 smallest areas, ranging from 3 46 cm ² to 40 cm ², were tested by exposing parts of the forehead The two larger areas consisted of the whole face and the upper half of the anterior body surface In Table I the minimum amounts of radiation which could be perceived as cold by each subject on each area are recorded together with the average figures for both subjects It is evident from this table that, as in the study with radiant heat, the threshold stimulus for cold decreased progressively with each increase in the area of the skin surface tested

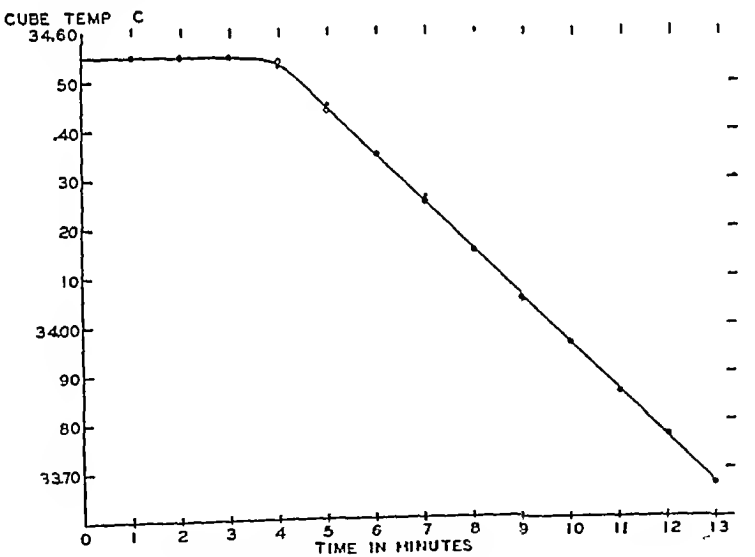


FIG. 2. CALIBRATION EXPERIMENT WITH SKIN TEMPERATURE RADIOMETER

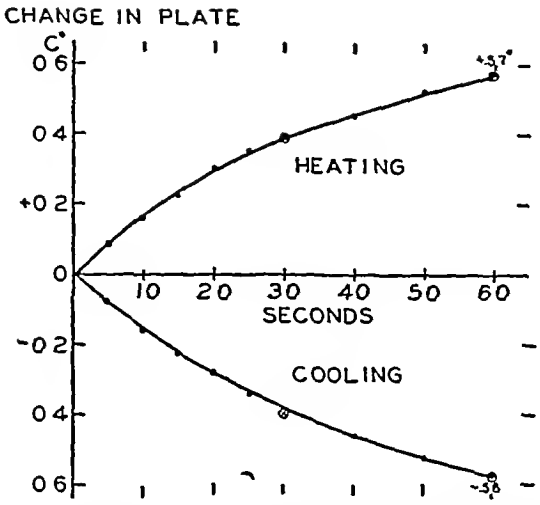


FIG 3 TEMPERATURE CHANGE PRODUCED IN A WARM, BLACKENED, COPPER DISC BY THE SAME INTENSITY OF HEAT AND COLD RADIATION

Dots represent measurements with a thermocouple soldered to the disc, circles, radiometer measurements

It was shown in the previous experiments that a radiation stimulus is a thermal stimulus, effective as a result of the skin temperature change which is produced In the last column in Table I, the changes in the temperature of the surface of the skin produced by the threshold stimuli are shown They likewise show decreasing values the larger the size of the area tested The relationship between the threshold stimulus (expressed as surface temperature change) and the size of the area tested is shown graphically in Figure 4 Because of the wide range of values the results are plotted in logarithms The curve is of the same type as that formed from the results of a similar experiment with non-penetrating infra-red radiation We were unable to obtain a sufficiently strong cold stimulus to test very small areas The threshold stimulus for a single end organ found by Bazett and McGlone (6) is shown added to our curve by an interrupted line

Within the range of radiation intensities used in these experiments the fall of skin temperature was directly proportional to the strength of the radiation applied for a constant time This is shown on Figure 1, in which skin temperature change is plotted against the strength of the radiation applied for 15, 30, 45, and 60-second periods The lines drawn between the points are straight and pass through the origin This result, which was also present in the experiments with heat, indicates that these temperature changes are independent of vasomotor effects and are produced by the absorption of the radiation There was a striking difference between the thermal effect of heat and cold radiation, cold changing the skin temperature much more than the same number of calories of heat This is shown on Figure 5, in which the results of experiments with heat and cold are plotted on the same scale The upper curve indicates changes caused by cold, lower line, changes resulting from heat

DISCUSSION

The unequal changes of skin surface temperature produced by identical amounts of heat and

TABLE I

Minimum stimuli for various sized body areas

Area		Subject I	Subject II	Average	Skin temperature elevation
Location	Size	gm cal / sec /cm²	gm cal / sec./cm²	gm cal./ sec /cm²	Rate
	cm²				*°C per second*
Forehead	3 46	0 0013 0 0013 0 0013	0 0012 0 0014 0 0013	0 0013	0 019
Forehead	7 08	0 00094 0 00097 0 00094 0 00094	0 00094 0 00095 0 00092	0 00094	0 013
Forehead	10 0	0 00082 0 00079 0 00072 0 00072	0 00082 0 00077 0 00072 0 00074	0 00076	0 011
Forehead	14 5	0 00064 0 00072 0 00063	0 00059 0 00065 0 00068 0 00062	0 00065	0 009
Forehead	23 8	0 00046 0 00036 0 00051 0 00047	0 00051 0 00051 0 00048 0 00050	0 00048	0 007
Forehead	40 0	0 00046 0 00043	0 00041 0 00038	0 00042	0 006–
Entire face	197	0 00033 0 00032	0 00036 0 00033	0 00033	0 004
Face and chest	1,680 (I) 1,940 (II)	0 00032 0 00027	0 00026 0 00022	0 00027	0 004–

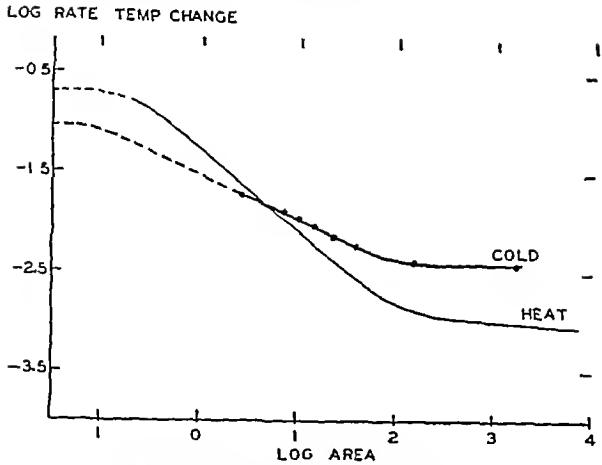


FIG. 4 COMPARISON OF HEAT AND COLD SENSATION AS FUNCTIONS OF TEMPERATURE CHANGE AND EXPOSED AREA

cold radiation was an unexpected and at first perplexing finding The experiments in which the temperature of the blackened copper plate was measured after warm and cold radiation indicated clearly that the measurements were accurate and that the observed phenomena were not owing to an error in technique. Vasomotor changes seemed to be eliminated as a cause by the linear relationship found between surface temperature change and the quantity of the heat or cold radiation absorbed Bazett and McGlone (6) had reported that cold is conducted into the skin more slowly than heat and it seemed that this might explain our results on a purely physical basis At the room temperature in which we worked the skin surface was colder than the deeper structures and heat was constantly flowing out A simple experiment with artificial material was devised to stimulate the conditions in the skin

A piece of beef muscle 7 mm thick was pasted on one side of a Leslie cube. The water in the cube was maintained at 37.20° C. It was then irradiated for periods of 30 and 60 seconds with equal amounts of heat and cold, and the change in its surface temperature after each period was measured with the radiometer in the same way that the temperature of the skin surface was measured after irradiation The results are shown on Figure 5 together with the changes in skin surface temperature produced by the same amounts of radiation The lower curve is the temperature change caused by heat radiation, the upper curve by cold radiation Almost exactly the same temperature changes occurred on the meat and on the skin surface. The difference observed between the thermal effect of warm and cold radiation therefore seems to depend upon the thermal properties of tissue and is entirely independent of the blood flow in the skin This

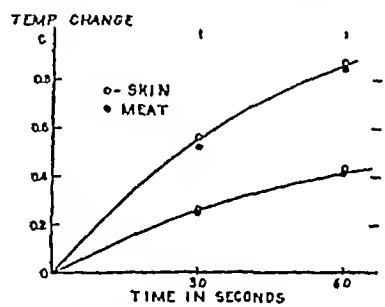


FIG. 5 TEMPERATURE CHANGES I
AMOUNTS OF HEAT AND COLD R
COLD RADIATION, LOWER CURVE, H

phenomenon occurs only during short periods of irradiation, for the circulation in the skin is altered after one or two minutes' exposure and for long periods like amounts of heat and cold radiation do not cause equal and opposite skin temperature changes

Because of the phenomenon just discussed, it is obvious that heat and cold sensation cannot be compared on the basis of radiation intensities and that comparison is possible only when the change of the skin temperature is considered as the stimulus In our results the actual temperature changes at the end organs are not known, for they will depend on thermal conduction into the skin We have contrasted the skin surface temperature increase or decrease associated with minimal sensations of warmth and cold for a number of different sized areas This is graphically shown on Figure 4 Analysis of these curves reveals certain interesting differences between the perception of warmth and cold In the region of large areas the curves are horizontal and further increase in the size of the area tested, although increasing the number of end organs stimulated, fails to reduce the threshold stimulus The minimum temperature change has been reached to which end organs will respond This threshold for cold end organs is a fall of skin temperature of 0 004° C per second and is much greater than the threshold for warm end organs of 0 001° C per second

The curve for cold sensation slopes more gradually and eventually crosses the curve for heat sensation so that in the region for small areas the sensory threshold for warmth is higher than the sensory threshold for cold This, together with Bazett and McGlone's value for the threshold for

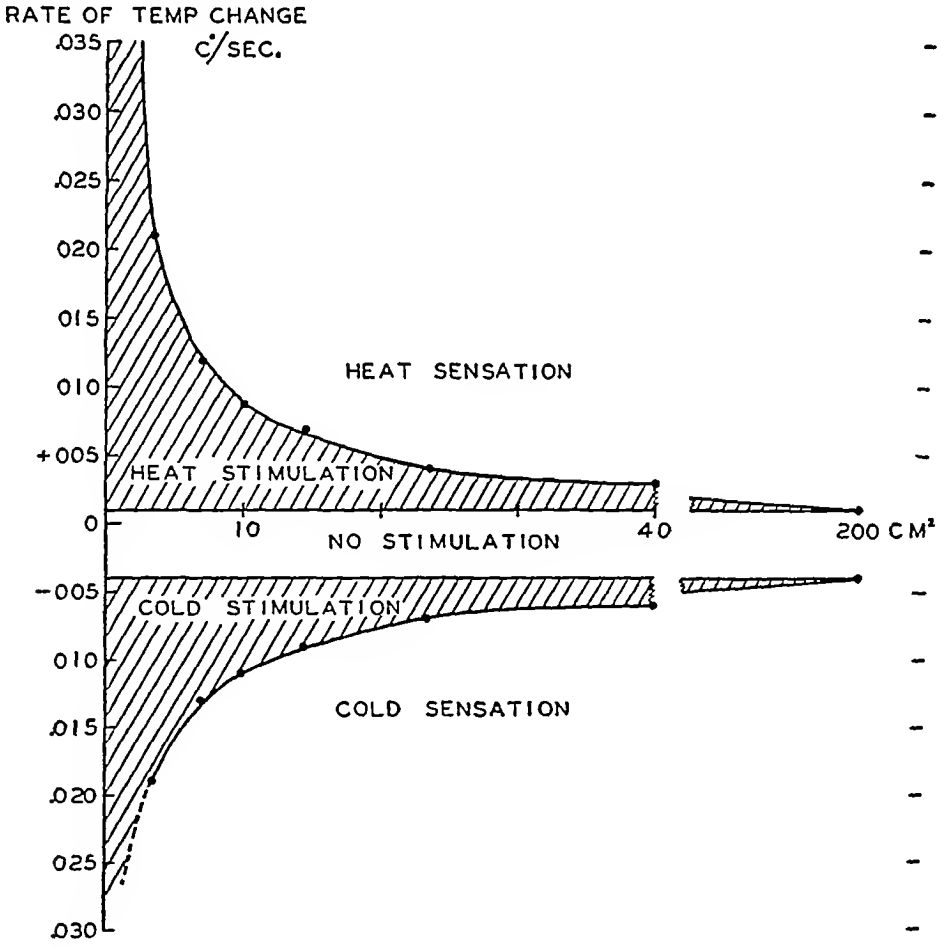


FIG 6 TEMPERATURE SENSATION AS A FUNCTION OF THE RATE OF SKIN TEMPERATURE CHANGE AND AREA EXPOSED

(See text for detailed description)

a single end organ, indicates that the number of cold endings is greater than that of the heat endings and that responses from warm end organs are summated more effectively than responses from cold end organs

These findings strongly support the belief that separate end organs respond to increases and to decreases of skin temperature. An elevation of skin surface temperature of 0 001° C per second activates heat receptors, a fall of 0 004° C per second, cold receptors These responses take place within a range of skin temperatures of several degrees, the exact limits of which were not determined It is entirely possible for an object remaining at constant temperature to stimulate warm receptors when the skin is cold and cold receptors when the skin is warm. The warmness or coldness of any object is therefore a physiological property dependent entirely upon its effect in altering skin temperature. This effect can be brought about by vasomotor change, by vaporization, draughts or the conduction or radiation of heat or cold to the skin There is no evidence from these experiments to support the contention of Nafe and Wagoner (7) that temperature sensation is secondary to vasomotor responses to thermal stimuli

To summarize these experiments on the perception of heat and cold by the human organism in a comfortable environment, we have added Figure 6 The upper half of the chart represents the change in sensory threshold for heat over a wide range of areas on the face and anterior thorax, the lower half, the change for cold over the same areas The regions external to the curves represent conditions under which sensation of warmth or cold is experienced the ruled regions, conditions under which end organs are responding but no sensation is evoked The clear portion between the ruled areas show the degree that the skin temperature can be raised or lowered without activating thermal receptors

SUMMARY

Using the radiation technique previously described, the end organs in the skin which are sensitive to cold were stimulated The term "cold" radiation was defined The source of cold radiation was a block of CO_2 snow, the rays from which were concentrated onto the skin by a large silver cone The foreheads of six subjects were tested and the individual sensitivity to cold radiation found to be approximately the same Two subjects were studied in detail by determining the minimum stimulating intensities of cold radiation for areas of different sizes The thermal changes produced at the skin surface by these radiations were measured to ± 0 005° C with a radiometer

CONCLUSIONS

1 The number of cold end organs per unit area is greater than the number of heat endings and they are nearer to the skin surface.

2 On the forehead, spatial summation of cold sensation follows the same pattern as for heat sense but the summation is poorer

3 The threshold of thermal stimulation for a cold end organ is a fall in skin temperature of 0 004° C per second

4 Cold radiation produces about twice the rate of change in skin temperature, calorie for calorie, as does heat radiation

5 Temperature sensation does not depend upon vascular changes in the skin

BIBLIOGRAPHY

1 Oppel, T W and Hardy, J D Studies in temperature sensation. I. A comparison of the sensation produced by infra red and visible radiation. J Clin. Invest., 1937, 16 517

2. Oppel T W and Hardy J D., Studies in temperature sensation. II The temperature changes responsible for the stimulation of the heat end organs. J Clin. Invest., 1937 16 525

3 Hardy J D and Oppel, T W., Studies in temperature. III. The sensitivity of the body to heat and the spatial summation of the end organ responses J Clin. Invest., 1937 16, 533

4 Goldscheider, A., Thermoreceptoren. Vol. 9 Pt. 1 Handbuch der normalen und Pathologischen Physiologie. Berlin, Springer 1926

5 Levine, H. A. and Dallenbach, K. M., Adaptation of cold spots under continuous and intermediate stimulation. Am. J Psychol., 1936 48, 485

6. Bazett, H
II The
tions of
Psychiat.

7 Nafe, J P
thermal :

of the blood vessels of the skin of a contralateral hand J Psychol, 1936, 2, 461

8 Herrington, L P, Winslow, C E A, and Gagge, A. P, The relative influence of radiation and convection upon vasomotor temperature regulation. Am J Physiol, 1937, 120, 133

9 Jung, R., Doupe, J, and Carmichael, E. A., Shivering A clinical study of the influence of sensation. Brain, 1937, 60, 28

10 DuBois, E F., The mechanism of heat loss and temperature regulation The Lane Medical Lectures, Stanford University Press, 1937

THE COAGULATION DEFECT IN HEMOPHILIA. STUDIES ON THE REFRACTORY PHASE FOLLOWING REPEATED INJECTIONS OF GLOBULIN SUBSTANCE DERIVED FROM NORMAL HUMAN PLASMA IN HEMOPHILIA[1]

By FREDERICK J POHLE AND F H L. TAYLOR

(From the Thorndike Memorial Laboratory Second and Fourth Medical Services (Harvard), Boston City Hospital and the Department of Medicine Harvard Medical School Boston)

(Received for publication August 29, 1938)

It has been suggested that the defect in coagulation of blood in hemophilia resides in a globulin fraction of the plasma (1, 2) Globulin substance, prepared by isoelectric precipitation from citrated, cellular-free, normal human plasma, posseses marked clot-accelerating properties for hemophilic blood *in vitro* and *in vivo* There is a prompt decrease in the blood coagulation time when a saline suspension of this material is injected either intravenously or intramuscularly into hemophilic subjects The coagulation time remains low following such injections for approximately 6 hours when it commences to return toward the pre-injection level Attempts to *maintain* a *reduction* of the coagulation time with repeated injections of globulin substance led to the discovery that a refractory phase occurred after several injections had been given (2) During this phase the coagulation time was little affected by repeated injections although it was demonstrated that the concentration of this clot-accelerating material progressively increased in the circulating blood of the patient. Recovery from this refractory state was always complete within 24 hours after the last injection of globulin substance The present communication reports further studies concerning the refractory period and offers an explanation of certain aspects of this phenomenon.

METHODS

Coagulation time The method by which the coagulation time of venous blood was determined has been described elsewhere (2)

Control period The investigation was carried out on four hemophilic patients between the ages of 18 and 48 years who had been under observation in this clinic for several years The shortest blood coagulation time for these four patients was 40 minutes and the longest was 180 minutes Prior to each set of observations a control period of 48 hours was established If the coagulation time of the blood fluctuated appreciably during this period no test observations were made of that subject. Patients who had had recent hemorrhages were excluded from this study

Preparation of globulin substance The globulin substance was prepared from citrated normal human plasma in the same manner as previously described (2) In all experiments the dried material was suspended in the same volume of 0 85 per cent sodium chloride solution as the volume of plasma from which it was derived rendered sterile and free of particulate matter by Berkefeld filtration Each lot of globulin substance, so prepared, was shown to have maximum clot accelerating properties for hemophilic blood *in vitro* (2) before it was used parenterally

Standard test dose A standard test dose of 65 cc. of plasma or an equivalent amount of saline suspension of globulin substance was used for *in vivo* studies The standard test dose of globulin substance contained approximately 300 mgm. of the dried material

Preparation of dried material by the Flosdorf-Mudd technique In certain instances, plasma or the freshly prepared, moist precipitate of globulin substance was dried by the lyophilic process as described by Flosdorf and Mudd (3) This porous dry material prepared from either source was redissolved in an amount of distilled water equal in volume to that of the original plasma

[1] The expenses of this research were defrayed in part by a gift to Harvard University from Smith, Kline, and French Laboratories of Philadelphia.

EXPERIMENTAL

The effect of repeated intravenous injections of plasma In view of the fact, shown by many observations, that a refractory phase followed repeated injections of globulin substance into hemophilic patients (Figure 1), it was necessary to observe the effects of repeated injections of the parent plasma [2] Three hemophilic subjects were given a standard test dose of citrated normal human plasma intravenously, repeated four times at 6-hour intervals The results obtained in one typical set of observations are presented in Figure 2 As shown in Figure 2, the blood coagulation time was maintained near normal values, and throughout the entire period of observation there was *no suggestion of the development of a refractory period following any injection of plasma*

The effect of dried plasma upon the coagulation time of hemophilic blood in vitro Normal citrated human plasma was dried in open dishes at room temperature in a current of air created by an electric fan The dry material, so obtained, was suspended in the same volume of distilled water as the original volume of the plasma, centrifuged, and then its clot-accelerating properties for hemophilic blood tested *in vitro* Normal plasma has been shown to reduce the coagulation time of hemophilic blood in a quantitative manner (1, 2) There was a marked loss of these properties associated with the type of desiccation described, as shown by Table I

TABLE I

Effect of a solution of air-dried, citrated, normal human plasma on the coagulation time of hemophilic blood in vitro

	Coagulation time
	minutes
2 cc hemophilic blood	43 5
2 cc hemophilic blood + 0 01 cc suspension of dried plasma	43 5
2 cc hemophilic blood + 0 03 cc suspension of dried plasma	41 0
2 cc hemophilic blood + 0 05 cc suspension of dried plasma	42 0
2 cc hemophilic blood + 0 1 cc suspension of dried plasma	35 0
2 cc hemophilic blood + 0 2 cc suspension of dried plasma	28 5

Normal citrated plasma was then dried by the lyophilic process This material when redissolved in distilled water showed maximum clot-promoting properties for hemophilic blood *in vitro* (Table II) These observations indicated that the clot-accelerating properties of plasma

[2] All plasma referred to in this communication has been passed through a Berkefeld V filter

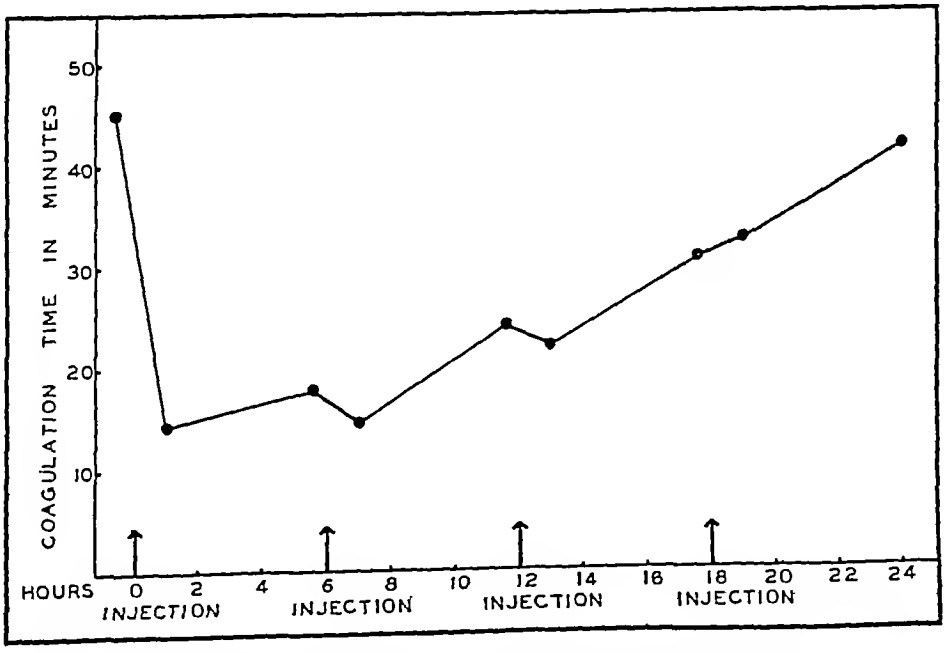


FIG 1 EFFECT OF MULTIPLE INTRAMUSCULAR INJECTIONS OF GLOBULIN SUBSTANCE ON THE COAGULATION TIME OF THE BLOOD IN HEMOPHILIA

TABLE II

Effect of lyophilized citrated normal human plasma on the coagulation time of hemophilic blood in vitro

	Coagulation time
	minutes
2 cc. hemophilic blood	70 0
2 cc. hemophilic blood + 0 01 cc. lyophilic plasma	21 0
2 cc. hemophilic blood + 0 03 cc. lyophilic plasma	12 0
2 cc hemophilic blood + 0 05 cc. lyophilic plasma	10.5
2 cc. hemophilic blood + 0 1 cc. lyophilic plasma	6.5
2 cc. hemophilic blood + 0 2 cc. lyophilic plasma	4.5

were not disturbed by the freezing and drying process used in this technique.

The effect of parenteral injections of lyophilized plasma. A single intravenous or intramuscular injection of lyophilized plasma redissolved in 65 cc. of distilled water was given to the same hemophilic patient on four different occasions In each instance there was a prompt reduction in the coagulation time of the blood to normal or near normal values within one half hour This reduction in the coagulation time was maintained for approximately six hours after which it gradually returned to the pre-injection level. Occasionally, the patients experienced some local pain following the intramuscular injection but no hematomas or general systemic reactions occurred

A standard test dose (65 cc.) of a solution of lyophilized plasma was given intravenously four times at 6-hour intervals to a hemophilic patient. The results were entirely comparable to those shown in Figure 2 The shortened coagulation time was maintained and *a refractory period did not develop* following any of the injections The observations were repeated except that the plasma was administered intramuscularly The results were again entirely similar

The effect of drying globulin substance by the Flosdorf-Mudd technique Globulin substance was prepared in the usual manner except that immediately after centrifugation the moist precipitate was frozen and dried under vacuum by the lyophilic process This material was then ground, stored in a desiccator over calcium chloride, and as required suspended in the requisite amount of saline solution The clot accelerating properties of this preparation for hemophilic blood were tested *in vitro* (Table III) and *in vivo* Globulin substance dried in this manner retained all of its potency

One hemophilic subject was given intramuscularly a standard test dose of globulin substance

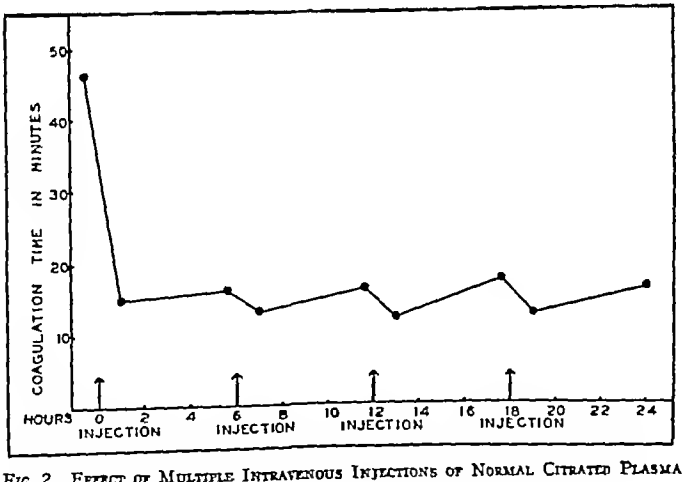


FIG 2. EFFECT OF MULTIPLE INTRAVENOUS INJECTIONS OF NORMAL CITRATED PLASMA ON COAGULATION TIME OF THE BLOOD IN HEMOPHILIA

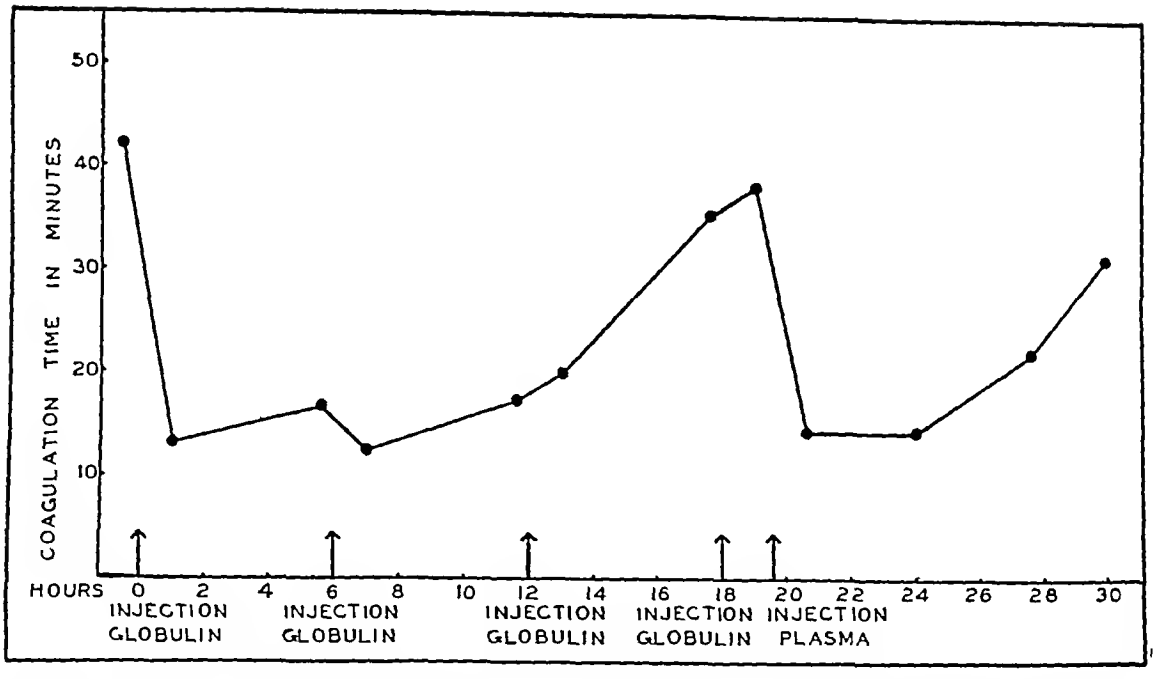


FIG 3 EFFECT OF A SINGLE INJECTION OF LYOPHILE PLASMA ON THE REFRACTORY PHASE FOLLOWING MULTIPLE INJECTIONS OF GLOBULIN SUBSTANCE IN HEMOPHILIA (ALL INJECTIONS INTRAMUSCULAR)

dried in this manner every 6 hours for 24 hours After the usual initial reduction in the coagulation time to normal values *a refractory phase developed* and further injections had little or no effect upon the coagulation time Data obtained were entirely similar to those expressed graphically in Figure 1 Blood platelet counts taken every 3 hours during this set of observations showed no fluctuations greater than normal

The effect of an intramuscular injection of lyophilized plasma upon the refractory phase One hemophilic subject was given a standard test dose of a saline suspension of globulin substance

TABLE III

Effect of a saline suspension of globulin substance (dried by the lyophilic process) on the coagulation time of hemophilic blood in vitro

	Coagulation time
	minutes
2 cc hemophilic blood	175 0
2 cc hemophilic blood + 0 01 cc suspension of globulin substance	19 0
2 cc hemophilic blood + 0 03 cc. suspension of globulin substance	8 0
2 cc hemophilic blood + 0 05 cc suspension of globulin substance	6 5
2 cc hemophilic blood + 0 1 cc suspension of globulin substance	5 0
2 cc hemophilic blood + 0 2 cc suspension of globulin substance	3 5

repeated four times at 6-hour intervals The usual refractory period developed after the first two injections and the blood coagulation time gradually returned to the pre-injection level The third and fourth injections were entirely without effect on the coagulation time of the blood Immediately after the fourth injection and at the height of the refractory period when further injections of globulin substance have been shown to be ineffective (2) this subject received a standard test dose of lyophilized plasma intramuscularly There was a sharp reduction in the blood coagulation time, and it appeared that the refractory phase had been abolished by this single injection of plasma (Figure 3)

COMMENT

The fact that many observations have shown that repeated injections of globulin substance into hemophilic patients produced a refractory phase, as indicated by failure of further injections of globulin substance to reduce the coagulation time of hemophilic blood (2), necessitated further investigations to determine the nature of this reaction Since the protein of the early preparations could have been denatured this condition might be considered a possible cause of the refractory phe-

nomenon. However, repeated injections of lyophilized globulin substance produced a similar refractory phase Flosdorf and Mudd (3) have shown that the lyophilic process does not modify the protein complexes, and therefore it is not likely that denaturization is responsible for the development of the refractory period

Earlier studies (2) demonstrated that the concentration of the clot-promoting factor progressively increased in the blood of the injected patient during the development of the refractory period It was clear, therefore, that the concentration of this material in the blood was such that a reduction of the coagulation time should have taken place if globulin substance was the only material deficient or modified in hemophilic blood From the rapidity of onset and recovery from the refractory state shown by the present and previous studies the phenomenon could not be a manifestation of an antigen antibody reaction. Furthermore, there has been no evidence produced in any of our investigations to suggest that a non coagulable phase occurs during the refractory phase or at any time after the administration of globulin substance.

The present observations show clearly that repeated injections of citrated normal human plasma or lyophilized plasma into hemophilic subjects maintains a reduced coagulation time of blood so long as the injections are continued (Figure 2) These data suggest that such plasmas contain certain substances active in blood coagulation not contained by preparations of globulin substance. This supposition is confirmed by the experience in one case in which lyophilized plasma was injected at the height of the refractory phase with a reduction of the blood coagulation time toward normal limits The nature of this substance or these substances is at present under investigation It is possible, however, from an inspection of the data presented in this and in a previous report (2), that a combination between globulin substance and this second substance or substances may play an essential role in the reduction of the coagulation time of hemophilic blood *in vivo* Since globulin substance, like citrated normal plasma, exerts its clot promoting effect on hemophilic blood in the test tube in a quantitative manner (Table III), it would appear that under these circumstances the second substance or substances was present in sufficient quantity to satisfy the requirements of increasing amounts of globulin substance However during the development of the refractory phase in the patient with hemophilia it is possible that the increasing concentration of globulin substance in the blood finally results in the exhaustion of this second substance. The abolition of the refractory phase with a single injection of plasma is probably due to an increase in titer of this second substance. To what extent the injection of such normal plasma will quantitatively affect subsequent injection of globulin substance is at present under investigation.

CONCLUSIONS

1 In hemophilia, repeated injections of lyophilized globulin substance as well as normal globulin substance produced a refractory period after the usual initial reduction in the coagulation time of the blood

2 Repeated injections of either normal human plasma or lyophilized plasma into hemophilics maintained a shortened blood coagulation time without, however, the development of a refractory phase

3 The refractory phase can be abolished at its height by a single injection of plasma

4 Both normal plasma and lyophilized plasma probably contain substances which play a role in the reduction of the coagulation time of blood *in vivo* and which are not present in globulin substance preparations

The authors gratefully acknowledge the assistance of Miss Nancy Marean.

BIBLIOGRAPHY

1 Patek, Arthur J., Jr., and Taylor, F H L. Hemophilia. II. Some properties of a substance obtained from normal human plasma effective in accelerating the coagulation of hemophilic blood. J Clin. Invest. 1937 16, 113

2. Pohle Frederick J., and Taylor F H L., The coagulation defect in hemophilia. The effect in hemophilia of intramuscular administration of a globulin substance derived from normal human plasma. J Clin. Invest., 1937 16, 741

3 Flosdorf Earl W., and Mudd, Stuart Procedure and apparatus for preservation in "lyophile" form of serum and other biological substances. J Immunol., 1935, 29, 389

A DIRECT METHOD FOR THE ESTIMATION OF SKIN DISTENSIBILITY WITH ITS APPLICATION TO THE STUDY OF VASCULAR STATES

By WILLIAM A. SODEMAN AND GEORGE E. BURCH

(From The Department of Medicine Tulane University of Louisiana and The Charity Hospital of Louisiana New Orleans)

(Received for publication July 19 1938)

Schade (1) first introduced objective quantitative methods for the study of the physical characteristics of human skin when in 1912 he described an elastometer designed to indicate skin elasticity Since that time many modifications of his method have appeared (2 3 4, 5 6 7 8) The present report is an attempt to study the physical characteristics of the skin not by Schade's method or its modifications which are essentially adaptations of the tonometer principle as applied to the eye but by an apparatus designed to measure the distensibility or 'stretchability' of the skin and thus to evaluate this physical property in normal and pathological physiology Applications of the method have come to light for the objective estimation of certain skin changes as influenced by therapeutic procedures where heretofore no satisfactory quantitative methods have been available

APPARATUS

The apparatus is illustrated in Figures 1 and 2. Figure 1 illustrates the apparatus in use while Figure 2 demonstrates the details of construction. It consists

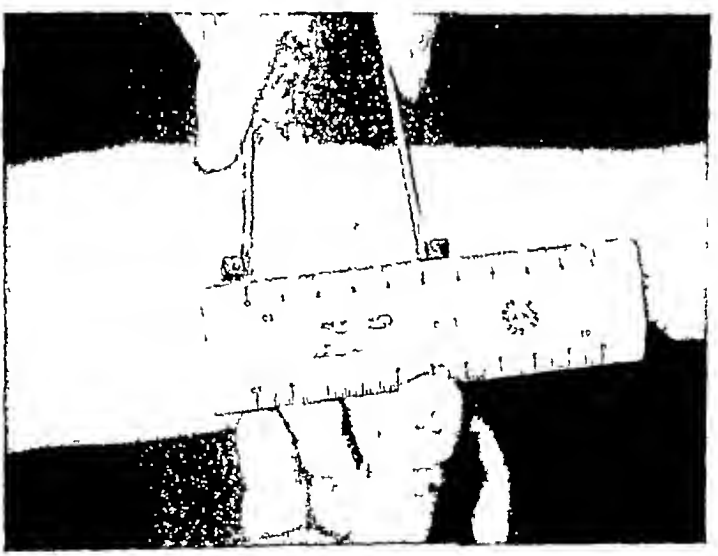


FIG. 1 THE APPARATUS IN USE

essentially of four parts a spring caliper *A* two bakelite cubes *B* and a brass adapter *C* The caliper consists of two *rigid brass arms b* which are sealed with DuPont household cement to the ends of a steel spring *a* The spring and length of the arms of the caliper were so chosen that when the knife-edges of the distal ends of the arms are 5 cm. apart these knife edges tend to separate with a force of approximately 100 grams

The caliper was calibrated as follows C of the caliper was fixed in a vise so

the other arm rested directly above that of the fixed arm A square piece of bakelite, 4 mm on edge and 2 mm thick, was grooved on one of the wide surfaces through the center and parallel to two edges The bakelite square was balanced with the groove articulating with the upper knife edge, and weights freely suspended from it by means of a loop of twine Weights were suspended in increments of 5 grams from 0 to 150 grams, at both ends so that there are two surfaces parallel to each other and 5 cm apart

In use, the skin surface is carefully cleansed with ether in order to remove the sebaceous secretions The bakelite cubes are then loosely sealed, with Johnson and Johnson's zinc oxide adhesive mass, into the notches of the adapter so that the grooved surfaces face each other 5 cm apart with the central groove perpendicular to the

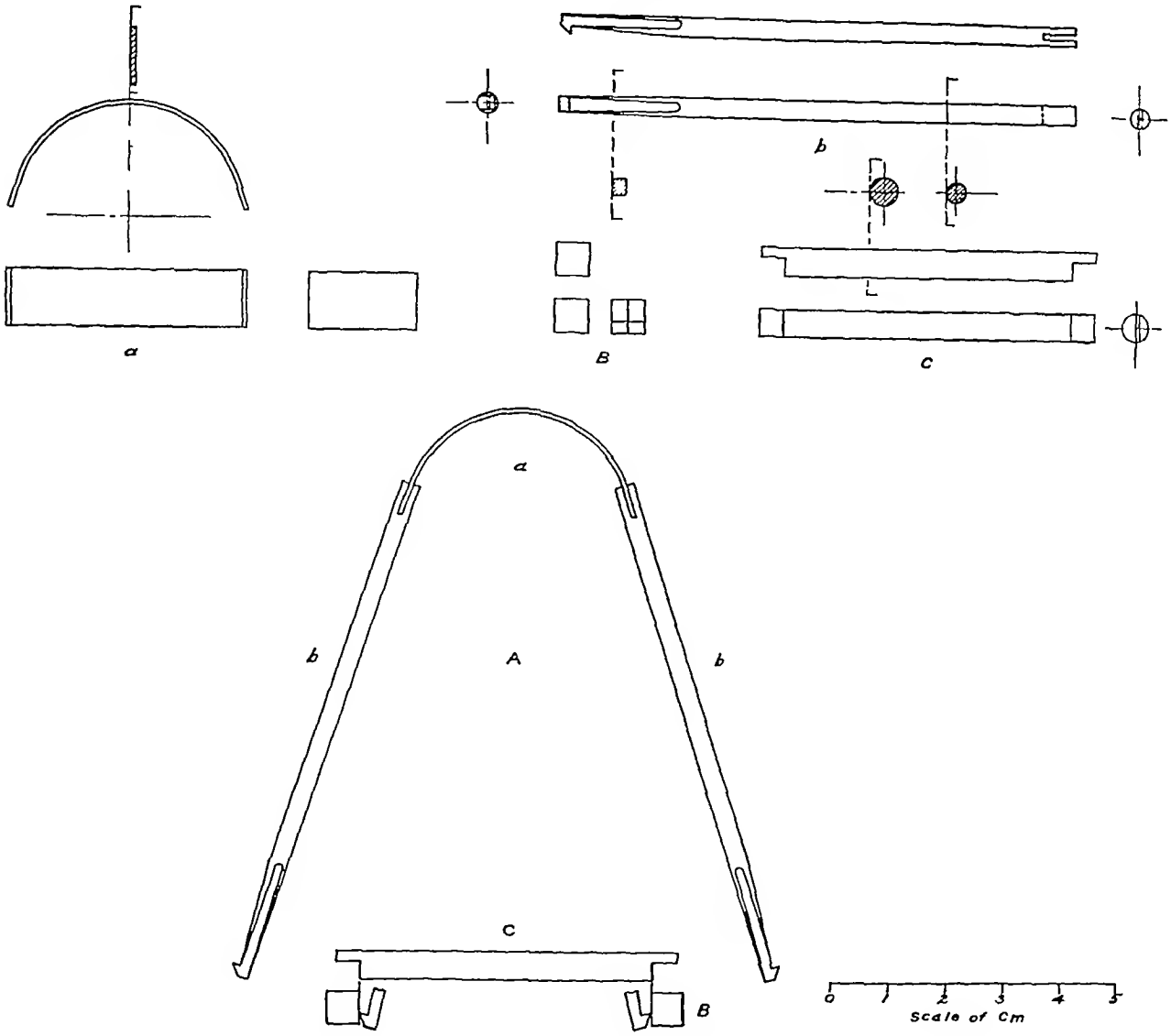


FIG 2 DETAILS OF CONSTRUCTION OF THE APPARATUS

adjustments being made to keep the line of force directly between the knife edges The distance between the knife edges was determined to 0 1 mm for each 5-gram increment by means of a hand lens and scale The results are illustrated in Figure 3 The formula for the line was found to be Force = 257 6 — 2 773 distance Recalibration of the instrument after three months of use failed to reveal any change in its characteristics

A surface in each of the cubes, *B*, is grooved as illustrated in Figure 2 The brass adapter, *C*, is notched adapter and the eccentric groove away from the adapter The cubes are then placed upon the previously prepared skin with the adapter above The cubes are then sealed to the skin with a thin layer of fresh collodion carefully applied to the junction of the cube and the skin on all sides except the grooved surfaces which face each other After five minutes have been allowed for the collodion to dry, the adapter is carefully removed leaving the bakelite cubes sealed to the skin with a distance of approximately 5 cm between them The distance between

the cubes is then accurately measured to 0.1 mm. with a scale. With the calipers held horizontal to the skin surface and closed so that the knife edges are less than 5 cm. apart the arms are then gently opened so that the knife edges articulate at the skin surface with the vertical grooves of the bakelite cubes. All pressure on the caliper arms is then released so that they open with their full force. The distance between the cubes is then remeasured. Care must be taken to maintain anatomical structures in the area studied in the same relative positions for determinations from patient to patient and for determinations from time to time in the same patient. Such a standard position for the part is extremely important as will be discussed later

Knowing the initial and final lengths of skin segment and the force exerted by the caliper one may express the distensibility of the skin in any convenient terms. We have chosen millimeters of stretch per centimeter of skin per 100 grams of force. The force exerted by the caliper is determined by referring to the graph of Figure 3 upon which the millimeter distance between the cubes when the pressure on the caliper arm is released, may be converted into force exerted in grams.

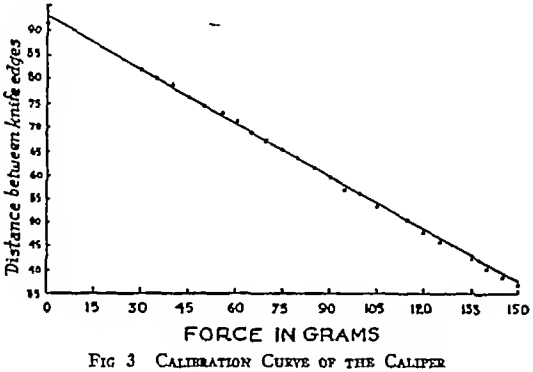


FIG 3 CALIBRATION CURVE OF THE CALIPER

The same data may be obtained from the formula already given.

RESULTS

Determinations were made on 13 normal subjects for the pretibial area, dorsum of the foot, midline of the abdomen below the umbilicus, volar surface of the forearm and the dorsum of the

TABLE I

Distensibility studies in 13 normal subjects

Subject number	Age	Sex	Color	Pretibial area *				Dorsum of foot				Abdomen				Volar surface of forearm				Dorsum of hand			
				D.B.S.	D.A.S.	T.D.	D.	D.B.S.	D.A.S.	T.D.	D.	D.B.S.	D.A.S.	T.D.	D.	D.B.S.	D.A.S.	T.D.	D	D.B.S.	D.A.S.	T.D.	D.
	years			mm.	mm.	mm.	mm. per cm. per 100 grams	mm.	mm.	mm.	mm. per cm. per 100 grams	mm.	mm.	mm.	mm. per cm. per 100 grams	mm.	mm.	mm.	mm. per cm. per 100 grams	mm.	mm.	mm.	mm. per cm. per 100 grams
1	22	F	W	50.8	51.0	4.3	0.79	50.8	54.3	3.4	0.63					51.0	58.5	5.5	1.06	51.7	60.0	8.3	1.77
2	21	F	C.	51.4	52.6	1.2	0.21	52.6	55.7	3.1	0.57	51.8	63.6	11.8	2.81	51.0	55.9	4.9	0.94	50.6	57.6	7.0	1.36
3	19	F	C.	51.5	52.3	0.8	0.14	51.0	55.5	4.5	0.87	52.0	63.3	11.3	2.58	52.4	56.8	4.4	0.84	57.8	60.3	8.5	1.83
4	18	F	C.	51.5	52.7	1.3	0.21	53.0	54.8	2.5	0.45	50.0	61.3	11.3	2.96	50.2	55.4	5.1	0.97	51.4	59.6	8.3	1.74
5	15	F	C.	51.4	53.7	2.3	0.43	50.6	54.1	3.6	0.64	51.2	56.7	5.5	1.30	51.3	56.3	5.0	0.96	50.5	58.3	7.4	1.53
6	24	F	C.	50.1	53.1	3.0	0.56	51.0	56.8	5.8	1.14	51.8	57.5	5.4	1.05	51.0	58.5	7.5	1.43	50.8	60.2	9.4	2.04
7	21	F	C.	50.5	52.4	1.5	0.29	51.1	54.0	2.9	0.53	50.0	57.8	7.8	1.59	51.9	55.2	3.4	0.64	50.7	55.3	4.6	0.87
8	30	M.	W	51.3	52.6	1.3	0.23	51.1	55.2	4.1	0.77					51.4	54.3	2.9	0.53	50.0	55.1	5.1	0.97
9	22	M.	W	50.4	52.1	1.7	0.30	51.5	53.7	1.9	0.34					51.5	55.1	3.6	0.67	51.2	54.8	3.6	0.66
10	22	M.	C.	50.9	52.6	1.7	0.30	51.1	54.7	3.6	0.66					52.0	56.3	4.3	0.82	49.6	55.2	5.7	1.11
11	23	M.	W	51.3	52.1	0.8	0.14	51.0	52.5	1.5	0.27					51.2	55.0	4.7	0.89	50.0	57.1	7.1	1.44
12	31	M.	W	51.6	53.5	1.8	0.32	50.5	53.1	2.6	0.47												
13	47	M.	W	51.1	52.7	1.6	0.29	50.8	5...9	2.1	0.37	50.9	59.1	8.3	1.72	51.4	56.1	4.7	0.89	50.5	54.9	4.3	0.78
	Mean						0.31				0.59				2.07				0.93				1.34
	Maximum						0.79				1.14				2.93				1.43				2.04
	Minimum						0.14				0.27				1.03				0.53				0.65

* D B.S = Distance before stretching D.A.S = Distance after stretching T D = Total D = Distensibility

hand, always in a direction parallel to Langer's lines of skin elasticity (9) These sites were chosen because of the frequency of edema and various dermatoses in these areas Results are

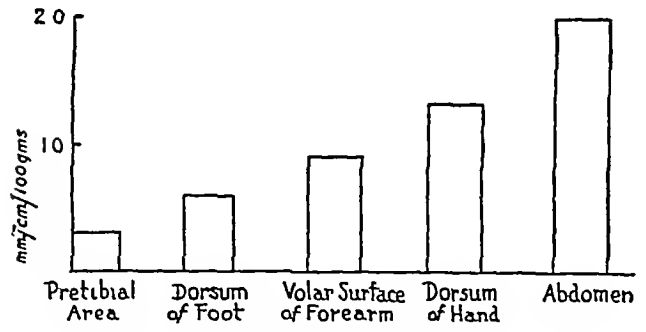


FIG 4 MEAN SKIN DISTENSIBILITY VALUES OF 13 SUBJECTS

illustrated in Table 1 The mean values were found to be 0 31, 0 59, 2 07, 0 93, and 1 34 mm per cm per 100 grams respectively The individual determinations may be found in Table I One can see that the skin is less distensible in the lower extremities This is graphically illustrated in Figure 4 These values vary inversely with the values previously determined for tissue pressure in the same areas (10) Determinations were repeated 15 times on the volar surface of the forearm of one subject at intervals varying from 6 to 72 hours Values varied from 0 87 to 0 98 mm per cm per 100 grams with a mean of 0.908 ± 0.006 and a standard deviation of 0.036 ± 0.004

The method has been applied to the study of certain abnormal states known to affect the skin These results are recorded in Table II and Figure 5 In eight patients with congestive heart failure, 21 determinations have been made. Patients

TABLE II

Distensibility values in various pathological states

Case number	Diagnosis	Age	Sex	Color	Date	Area studied	Distance before stretching	Distance after stretching	Total distance stretched	Distensibility	Remarks
		years					*mm.*	*mm*	*mm.*	*mm per cm per 100 grams*	
1	Congestive heart failure	66	F	W	Dec. 16, 1937	Left pretibial	51.2	52.4	1.2	0.20	Marked edema
					Dec. 20 1937	Left pretibial	51.1	52.2	1 1	0 19	Edema unchanged
					Dec. 20 1937	Left forearm	52.2	57.1	4.9	0 95	Moderate edema
					Dec. 22, 1937						Patient deserted
2	Congestive heart failure	43	M.	C	Jan. 12, 1938	Left pretibial	51 0	51 7	0 7	0.12	Marked edema
					Jan. 13, 1938	Left pretibial	51 6	51 8	0.2	0 03	Blebs developing
					Jan. 17 1938	Left pretibial	50 7	52 0	1.3	0.21	Edema decreasing
					Jan. 20, 1938	Left pretibial	51 9	54.2	2.3	0 41	Very slight pitting edema
					Jan. 21, 1938						Patient died
3	Congestive heart failure	69	M.	C.	Jan. 17, 1938	Left pretibial	50.5	51 6	1.1	0 19	Marked edema of long standing
4	Congestive heart failure	63	F	C	Jan. 20 1938	Left forearm	51.8	53.3	1.5	0.29	Marked edema
					Jan. 21, 1938	Left forearm	50 7	53.8	3 1	0.56	Edema decreasing
					Jan. 27, 1938	Left forearm	52.0	55.5	3.5	0 65	Edema decreasing. Patient died
5	Congestive heart failure	60	F	W	Jan. 21 1938	Left pretibial	51.5	53 6	2.1	0.38	Slight edema (a.m.)
					Jan. 21, 1938	Left pretibial	51.3	53 1	1.8	0.32	Edema increased (p.m.)
					Jan. 26, 1938	Left pretibial	50 7	52.7	2.0	0.36	Edema decreasing
6	Congestive heart failure	48	F	C	Feb 8, 1938	Right pretibial	51 0	51 9	0.9	0.16	Moderate edema
					Feb 9 1938	Right pretibial	50.3	51.6	1.3	0.23	Edema receding
					Feb 10, 1938						Patient died
7	Congestive heart failure	73	M.	C.	Feb 21, 1938	Right pretibial	50.5	51.2	0 7	0.12	Moderate edema
					Feb 22, 1938	Right pretibial	51.2	52.1	0 9	0.16	Edema unchanged
					Feb 24, 1938						Patient died
8	Congestive heart failure	60	F	C	Feb 28, 1938	Right pretibial	50 9	51 9	1 0	0 17	Moderate edema
					Mar 2, 1938	Right pretibial	50.8	51.8	1 0	0.17	Edema slightly decreasing
					Mar 5, 1938	Right pretibial	51.5	52.7	1.2	0.21	Edema slightly decreasing
9	Pernicious anemia with edema	60	M.	W	Dec. 20 1937	Right pretibial	51.5	52 7	1.2	0.21	Moderate edema
					Dec 23 1937	Right pretibial	50 6	51 7	1 1	0 19	Marked edema
					Dec. 30 1937	Right pretibial	51 0	51 0	0.0	0 00	Skin cracking. Bleb formation
					Feb. 14, 1938	Right pretibial	50.5	51.2	0 7	0 12	"Woody" edema
					Mar 14, 1938	Right pretibial	50 9	52.4	1.5	0.28	Skin softer Slight edema
10	Neuroblastoma with venous obstruction	6	F	C	Mar 15, 1938	Right pretibial	51 6	53.3	1 7	0.31	Marked edema
11	Urticaria	28	F	W	Feb. 25 1938	Dorsum of left hand	50.5	56 6	6 1	1.20	Wheals disappearing
12	Ascites, cause undetermined	57	M.	C	Feb 10, 1938	Abdomen	50.8	52.6	1.8	0.31	Marked ascites
					Feb 16, 1938	Abdomen	52.6	60.5	7 9	1.66	After paracentesis

TABLE II—*Continued*

Case number	Diagnosis	Age	Sex	Color	Date	Area studied	Distance before stretching	Distance after stretching	Total distance stretched	Distensibility	Remarks
		years					mm.	mm.	mm.	mm. per cm. per 100 grams	
13	Ascites, cause undetermined	65	M.	W	Feb. 21, 1938 Feb. 23, 1938	Abdomen Abdomen	51.5 51.0	53.1 53.2	1.6 2.2	0.28 0.39	Marked ascites Incomplete paracentesis
14	Ascites, heart failure	40	M.	C.	Feb. 23, 1938 Feb. 28, 1938	Abdomen Abdomen	51.2 50.2	55.4 58.3	4.1 8.1	0.77 1.69	Moderate ascites After paracentesis
15	Ascites, carcinomatosis	47	F	C.	Feb. 28, 1938 Mar 2, 1938	Abdomen Abdomen	50.6 51 7	53.2 57.8	2.6 6.1	0.47 1.22	Marked ascites After paracentesis
16	Peritonitis (ruptured tubo-ovarian abscess)	26	F	C.	Feb. 21 1938	Abdomen	51.4	57.8	6.4	1.29	Slight abdominal distension
17	Peritonitis, tuberculous	28	F	C.	Feb. 21, 1938	Abdomen	51.5	57.2	5.7	1 19	Slight abdominal distension
18	Senile skin (senile atrophy)	72	F	C.	Feb. 21, 1938	Volar surface of left forearm	50.8	55.2	4 4	0.83	
19	Occupational atrophy	31	M.	W	Jan. 5, 1938	Dorsum of right hand	50 4	54 4	4.0	0.73	
20	Occupational atrophy	65	M.	W	Feb. 21, 1938	Volar surface of right forearm	51.3	53.9	2.6	0.48	
21	Occupational atrophy	64	M.	W	Mar 9, 1938	Dorsum of right hand	51.6	54.6	3.0	0.55	
22	Allergic eczema	69	M.	W	Feb. 23, 1938	Volar surface of right forearm	50.8	52.3	1.5	0.37	
23	Scleroderma	12	F	W	Nov 9 1937 Nov 9 1937 Mar. 18, 1938	Left pretibial Right pretibial Left pretibial	50.0 50.0 50.4	50.8 53.5 51.5	0.8 3.5 1.1	0.13 0 45 0.19	Affected side Unaffected side Clinically unchanged
24	Scleroderma	38	F	W	Nov 11, 1937 Nov 11, 1937	Volar surface of right forearm Volar surface of left forearm	50.0 52.0	51.3 54.5	1.3 2.5	0.22 0 44	Markedly affected Moderately affected
25	Scleroderma	48	M.	W	Nov 20, 1937	Dorsum of right hand	50 6	53.3	2.7	0 48	
26	Scleroderma	42	F	W	Dec. 18, 1937 Dec. 18, 1937	Dorsum of left hand Dorsum of right hand	50.9 50.4	53.7 53.4	2.8 3.0	0.48 0.56	
27	Scleroderma	53	M.	W	Feb. 16, 1938 Feb. 16, 1938	Dorsum of right hand Dorsum of left hand	50.9 50.7	54.6 55.3	3.7 4.6	0.70 0.83	Slightly affected Slightly affected

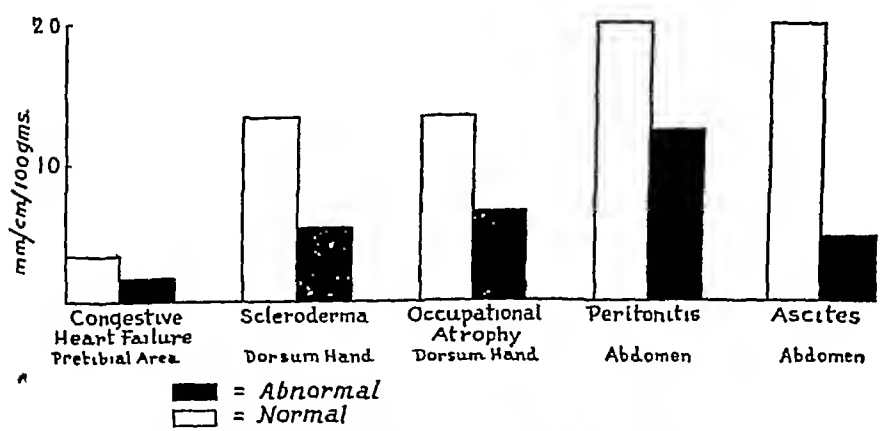


FIG. 5 COMPARISON OF MEAN DISTENSIBILITY VALUES IN NORMAL AND ABNORMAL STATES

were followed, when possible, through the course of the edema It was found that as the edema progressed the skin distensibility decreased and with recession of the edema distensibility tended to return to normal range Essentially the same results were found in the edema of pernicious anemia and that of venous obstruction resulting from neuroblastoma of the adrenal gland The data for the patient with pernicious anemia and edema, together with simultaneous determinations of tissue pressure, are illustrated in Figure 6

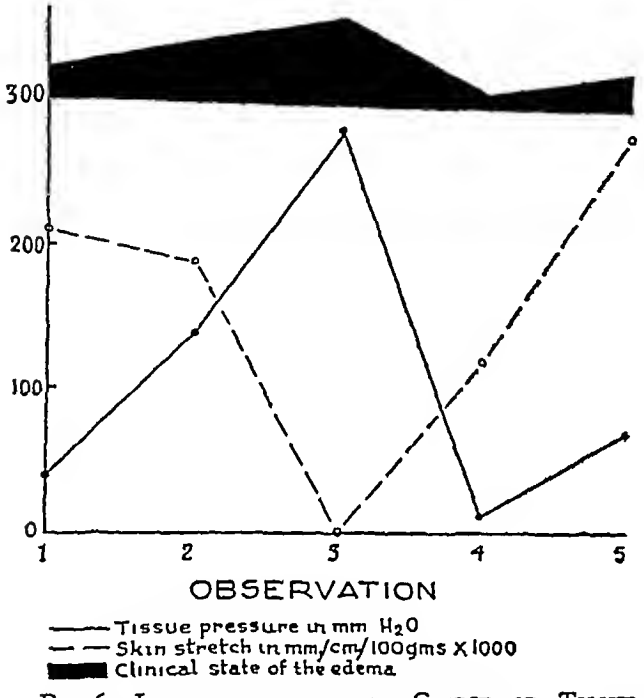


FIG 6 ILLUSTRATION OF THE COURSE OF TISSUE PRESSURE, SKIN DISTENSIBILITY, AND EDEMA IN A PATIENT WITH PERNICIOUS ANEMIA

The correlation of these values will be presented under *Discussion* Determinations of skin distensibility have been made upon the abdomens of four patients with ascites before and after paracentesis Marked differences were found as illustrated in Table II Similar, but less marked changes were found in two patients with peritonitis In certain dermatologic states (urticaria, senile atrophy, occupational atrophy, allergic eczema, and scleroderma) were observed definite changes in skin distensibility which correlated with the clinical state of the patient Individual and mean values may be found in Table II and Figure 5

DISCUSSION

For a number of years it has been realized (1) that an objective method for measuring skin changes in edematous states would have many important clinical applications With this is mind, Schade developed his elastometer His apparatus and the many modifications which have followed it are in reality mechanical palpators, capable of detecting changes too small to be picked up by ordinary clinical methods The method was also designed to measure skin elasticity, a phase of the apparatus most highly developed in the instrument of Inouye (3) While these methods are valuable in the measurement of certain aspects of tissue turgor, the influence of more deeply underlying structures introduces a variable which precludes their use for the measurement of skin distensibility and elasticity To obviate the influence of deep structures, as bone and muscles, we undertook to stretch the skin horizontally rather than to depress it Such a procedure reduces to a minimum the influence of structures below the dermis, and measures as far as possible without removal of the segment of skin the distensibility of the dermis and epidermis Since the normal skin tension of a given area varies with flexion and extension of adjacent joints and position of a part we found that standard positions were necessary for comparison of results Again one must be aware, in stretching a segment of the intact skin, that one not only stretches the segment under observation but also puts an oblique stress upon the skin lateral to the segment studied and pushes the skin distal to both ends of the segment Both of these influences enter into the results of each determination, but are relatively constant from individual to individual and more so in the same individual As long as the length of skin segment studied is the same, results are comparable from observation to observation and from patient to patient The only means of eliminating these influences is to remove the skin segment to be studied Such a procedure offers the disadvantage of necessary surgery, interference with innervation, circulation and the general normal physiology of the part studied, and the inability to do repeated determinations on the same segment during the progress of the disease

It should be made clear that our method measures the ability of the skin to stretch—its "stretchability," or distensibility We cannot measure elasticity in the intact skin in the pure physical sense, nor, indeed, can the methods of others Stress, or force per unit area, depends upon an exact knowledge of skin thickness which cannot be satisfactorily measured in the intact skin. Strain is, of course, easily measured. Our method measures the strain produced by a known total force which cannot be converted into force per unit cross sectional area This quantitative expression measuring strain per total force rather than strain per unit force lends itself admirably to the objective study of dermatologic changes, particularly in the clinic.

The constancy of our standards in a single site indicates either a constancy of quality and quantity of dermis and epidermis or an inverse relationship between the two, producing a constant relationship in distensibility Significant variation from the normal would indicate, therefore, that either the quality, the quantity, or both characteristics of the skin have undergone abnormal changes This is particularly exemplified in scleroderma where our results correlate with well known pathological changes of dense fibrosis in the dermis

The normal values for the areas studied have already been given (Table I) It is interesting to note the relatively marked distensibility of the abdominal skin and the relatively non-distensible skin of the lower extremities Just as quantitative variations in skin thickness are known to occur from person to person in one area, so do quantitative variations occur from area to area in the same individual The skin thickness is known to vary from approximately 0 37 mm. in the eyelids to 5 0 mm in the soles and palms Such variations not only involve the epidermis but the corium as well (11) The observed variations in skin distensibility may be accounted for either by qualitative and quantitative variations or by variations in skin tension in the parts studied We have previously reported (10) regional variations in tissue pressure which show an inverse relationship to the skin distensibility This relationship tends to show that the low skin distensibility in the lower extremities is owing, in part at least, to increased skin tension in these parts This regional variation may be of physiological significance in the prevention of edema of the feet on assuming the erect position.

The great distensibility of the abdominal skin is in accord with the marked physiological variations it must undergo, particularly in pregnancy We are at present engaged in a study of the changes in skin distensibility of the abdominal skin in and following pregnancy, especially in relationship to striae formation

Skin distensibility, as we have measured it, may be influenced in abnormal as well as in normal conditions, by at least three factors, (1) variations in skin tension, (2) changes in the quality of the dermal structures and (3) changes in the quantity of the dermal structures These factors may variably influence either the epidermis or the corium or both In our group of patients these variables have come into play, modifying the skin distensibility at times to a marked degree For example, in ascites caused by portal obstruction or tuberculous peritonitis (see Table II) the change in skin distensibility primarily results from a change in skin tension. Under such conditions, the skin is already stretched by the increased intra abdominal pressure and the 5 cm segment of skin measured for study does not represent 5 cm of undistended skin The application of the method to the distended skin indicates how much farther this stretched skin can extend with an additional force of 100 grams Therefore, any changes caused by the disease process would be accurately and quantitatively reflected in the measurements obtained In the instance of edema, not only does the factor of distension come into play, but there are changes in quality and quantity as well The distended, shiny skin of edematous parts exemplifies the first factor, changes in elastic fibers, long used as an explanation of pitting edema (12), are indicative of qualitative changes and actual swelling of the part with separation of dermal structures reduces the unit quantity of such elements As the disease progresses, all of the factors, interplaying as variables influence to a changing degree the skin distensibility The measurements of skin distensibility are quantitative expressions of this composite picture. The physiological sig-

nificance of these measurements in edema is appreciated when one becomes cognizant of the disturbed equilibrium of filtration and antifiltration With greater filtration as fluid accumulates, the tissues are stretched and become less distensible Tissue pressure then rises and tends to equalize the filtration pressure, acting as a limiting factor to the extent of the edema The loss of skin distensibility, as our results show, is one of the important limiting factors This effect is illustrated in Figure 6, where it may be seen that, with increasing edema and tissue pressure, there is a concomitant decrease in skin distensibility It is interesting to note that when the skin distensibility reached its limit for the force applied, the tissue pressure was greatest, the skin was beginning to crack, and bleb formation began The edema subsided almost completely and tissue pressure returned to normal limits and skin distensibility approached normal The final observation was taken when the edema increased slightly At this time the tissue pressure and skin distensibility increased An explanation for the absence of an inverse relationship between these two determinations at this time can only be conjectured Physical examination disclosed that skin texture was improving steadily following the period at which its elastic limits were reached The part was softer and less woody, in spite of the presence of edema This indicates a qualitative improvement in the skin characteristics Then, too, the blood hemoglobin was approaching normal, improving the tissue nutrition

The dermal changes found in scleroderma represent variations in all three factors Histologically, one can demonstrate qualitative and quantitative changes in the skin elements, particularly in the connective tissue of the corium The studies of Prinzmetal (13) illustrate definite changes in skin tension Our studies (14) tend to confirm this observation These abnormal variations in the physical characteristics of the skin apparently are directly proportional to the severity of the disease Again, since the distensibility measurements are dependent upon these physical dermal factors, the skin distensibility should vary inversely with the severity of the disease This was found to be true in the patients studied (Table II) In the parts more severely involved the distensibility values were found to be extremely low, with all values varying with the clinical state of the disease and less than normal for that part Such findings suggest the tremendous importance of this method of study for quantitatively evaluating the progress of scleroderma It also lends itself as a simple and rapid method for the early detection of results produced by, and the proper evaluation of, various therapeutic procedures The method also serves as a tool for the early diagnosis of scleroderma tous changes

The skin distensibility technic may be applied in the same manner to the study of skin changes in occupational atrophy and other dermatoses affecting the physical properties of the skin

SUMMARY AND CONCLUSIONS

A simple and accurate method for the measurement of skin distensibility is described

The normal mean values for the pretibial area, dorsum of the foot, midline of the abdomen below the umbilicus, volar surface of the forearm and dorsum of the hand were found to be 0 31, 0 59, 2 07, 0 93, and 1 34 mm per cm per 100 grams, respectively The regional variation disclosed less distensible skin in the lower extremities

Edema, certain vascular diseases, and some dermatoses were found to produce changes in the normal skin distensibility As edema progressed the skin distensibility decreased and with recession of the edema, distensibility tended to return to normal range The loss of skin distensibility was found to be an important limiting factor in edema formation In urticaria, senile atrophy, occupational atrophy, allergic eczema, and scleroderma were observed definite changes in skin distensibility which correlated with the clinical state of the patient In such diseases the method lends itself as a simple and rapid procedure for the early detection of results produced by and the proper evaluation of various therapeutic procedures

BIBLIOGRAPHY

1 Schade, H, Untersuchungen zur Organfunction des Bindegewebes I Die Elasticitätsfunction des Bindegewebes und die intravitale Messung ihrer Störungen. Ztschr f exper Path u Therap, 1912, 11, 369

2. Kunde, M M., Edema. I Correlation of elastometer readings disappearance time for intradermally injected salt solution, urinalysis and nitrogen retention of the blood in edema. Arch. Int. Med., 1926, 38, 57
3. Inouye, K., Dermoelastometry I A new dermoelastometer Acta scholae med. univ imp in Kioto 1931–32, 14, 229
4 Inouye, K. Dermoelastometry II. Clinical investigation into dermoelastometry Acta scholae med. univ imp. in Kioto 1931–32, 14, 232.
5 Inouye, K., Dermoelastometrie research The effect of standing work. Acta scholae med. univ imp in Kioto, 1931–32, 14, 276.
6. Trendtel, F., Elastometrische Untersuchungen an Kindern. Ztschr f d. ges. exper Med., 1926, 49, 327
7 Schwartz, A. B., The clinical study of edema by means of the elastometer Arch Int. Med., 1916, 17, 396
8 Rondelli, U., Sulla misura della "tensione cutanea." Minerva med., 1934 1, 810
9 Davis J S., Plastic Surgery Its Principles and Practice. P Blakiston s Son and Co Philadelphia, 1919, p. 25
10 Burch, G E. and Sodeman W A., The estimation of the subcutaneous tissue pressure by a direct method. J Clin. Invest. 1937, 16 845
11 Spalteholz W., Hand Atlas of Human Anatomy J B Lippincott. Philadelphia, 1923 4th English ed.
12. Wiggers C. J., Physiology in Health and Disease. Lea and Febiger Philadelphia, 1934, p. 828
13 Prinzmetal M., Studies of the mechanism of circulatory insufficiency in Raynaud s disease in association with sclerodactylia. Arch. Int. Med., 1936 58, 309
14 Sodeman, W A. and Burch G E., The tissue pressure in subcutaneous edema. Am. J M Sc., 1937, 194, 846.

CARBOHYDRATE TOLERANCE AFTER PROTAMINE INSULIN ITS BEARING ON THE PHYSIOLOGY OF INSULIN SECRETION [1]

By HENRY T RICKETTS

(From the Department of Medicine University of Chicago Chicago)

(Received for publication August 9, 1938)

The question of whether or not the behavior of the blood sugar in the normal organism after a carbohydrate meal depends upon the secretion of extra insulin is one which cannot be answered conclusively until a method is made available for measuring the amount of insulin in the blood stream There is, however, considerable indirect evidence favoring the theory that normally the ingestion of carbohydrate stimulates the secretion of insulin by the pancreas (1)

In 1934 doubt was cast on the validity of this theory by the work of Soskin, Allweiss and Cohn (2), who showed that in depancreatized dogs receiving a constant intravenous injection of glucose and insulin so adjusted as to maintain the blood sugar normal and level, an added dose of glucose gave a normal sugar tolerance curve provided the liver was intact Extra insulin, therefore, was neither available nor required, and it was concluded that the pancreas is not essential to the production of a normal glucose tolerance curve, though, on the basis of further evidence, the liver was deemed necessary

The first hint that these results, together with their implications, might apply only in the rather special conditions under which they were obtained came from clinical investigations of diabetic patients treated with protamine insulin It has been the common experience of physicians that in severe diabetes a daily dose of protamine insulin which would render the blood sugar normal before breakfast or during the night would often fail to prevent hyperglycemia after meals (3) Such experiences constitute a parallel fairly close to, but more physiologic than, the experiments with intravenous insulin and glucose in depan creatized dogs It is apparent in such cases that the constant supply of insulin derived from the subcutaneous depot of protamine insulin, although it is able to take care of the endogenous carbohydrate metabolism, needs to be augmented at meal time with extra insulin if the blood sugar is to be kept within normal limits On the other hand, it is well known that in cases in which the disease is relatively mild the blood sugar can be controlled at all times with protamine insulin alone.

Suggestive as the experiences with severe diabetics were, there remained the possibility that the abnormal behavior of the blood sugar after meals, especially after breakfast, might be owing to the beginning exhaustion of the protamine insulin given the previous morning or to the slow action of the dose given on the morning in question. It was decided therefore, to obtain blood sugar curves in such patients under more carefully controlled conditions

Hospitalized patients with diabetes of varying degrees of severity but well regulated with one or the other type of insulin were given, in the morning or evening of one day, a dose of protamine insulin which during the entire morning of the following day, without food, was shown to maintain the blood sugar at a constant normal level Several days later or earlier in each case, and under identical circumstances, a similar experiment was performed, this time giving the breakfast allowed by the patient's usual diet. Determinations of the blood sugar [2] were made before and at intervals after the meal In addition, comparable experiments were carried out in two chronically depancreatized dogs and in one normal dog The blood sugar time curves and pertinent clinical data are given in the accompanying charts

DESCRIPTION OF CHARTS

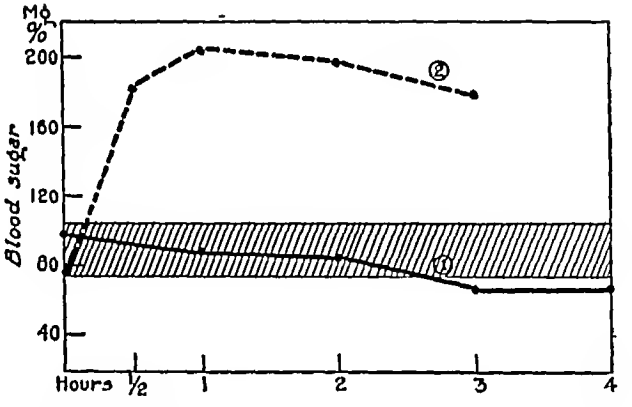
Severe diabetes

Patient E V (Figure 1) Thirty five units of protamine insulin given at supper time on *June 15 1937*

[1] Presented in part *before the American* Physiological Society, April 1938, at Baltimore, Maryland.

[2] Analyses were made on capillary blood by the method of Miller and Van Slyke (4)

Patient E V, No 176554 Severity Severe Sex F
Age 31 Duration 9 mos



Curve 1 6-16-37 No food 35 u P I given preceding evening

Curve 2 6-18-37 After usual breakfast containing 33 gm. CHO 35 u P I given preceding evening

Complications Admitted June 7 with ketosis but no acidosis

Course Urine contained less than 12 gm sugar per 24 hrs after second day, less than 4 gm. after ninth day Occasional insulin reactions

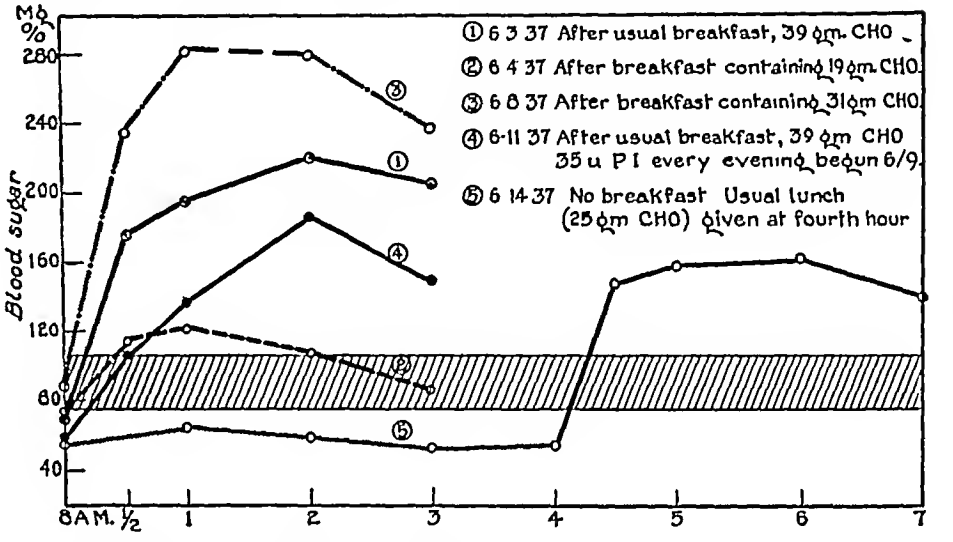
Diet C 100, P 60, F 150, including bedtime feeding

Insulin Regular insulin 65 to 30 u given in 4 to 2 doses daily except just before and on test days Eventual requirement 40 u P I daily

FIG 1 BLOOD SUGAR CURVES AFTER PROTAMINE INSULIN IN A DIABETIC PATIENT WITH AND WITHOUT FOOD

Cross-hatched areas in this and succeeding figures represent the normal range of blood sugar

Patient R B, No 149875 Severity Severe Age 9 Sex M Duration 15 mos



Complications Infectious mononucleosis two weeks before first test No fever, symptoms or increase in insulin requirement for 10 days before first test.

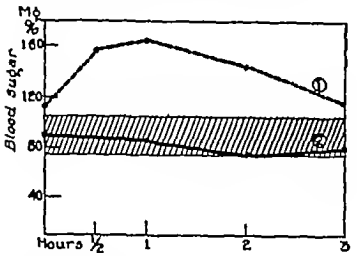
Course Occasional asymptomatic hypoglycemia Glycosuria 8–12 gms per 24 hrs

Diet C 130, P 89, F 143, including bedtime feeding

Insulin 35 u P I every morning until June 9 when evening administration was begun Eventual requirement 5 R + 30–35 P I daily

FIG 2 BLOOD SUGAR CURVES AFTER PROTAMINE INSULIN IN A DIABETIC PATIENT WITH AND WITHOUT FOOD

Patient B S No 155131 Severity Moderate to Severe. Age 65 Sex F Duration, 7 yrs.



Curve 1 10-21 37 After usual breakfast, 24 gm. CHO 50 u. P.I. given precedmg morning also this A.M.

Curve 2. 10-23 37 No food. 50 u. P.I given pre ceding morning

Complications Hypertension, Arteriosclerosis

Course Sugar-free throughout. No hypoglycemia.

Diet C 120, P 70 F 139 (including bedtime feeding)

Insulin 50 u. P I. given every morning ½ hr before breakfast except on morning of fast. Eventual requirement 45 u. P.I daily

FIG. 3 BLOOD SUGAR CURVES AFTER PROTAMINE INSULIN IN A DIABETIC PATIENT WITH AND WITHOUT FOOD

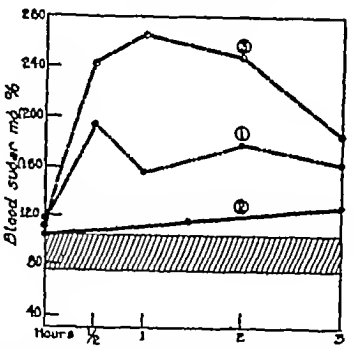
kept the blood sugar normal during the entire morning of June 16 when no breakfast was allowed (Curve 1) The same dose of protamine insulin given at supper on June 17 failed to prevent hyperglycemia after a breakfast containing 33 grams of carbohydrate on June 18 (Curve 2)

Patient R B (Figure 2) Curves 1 2, and 3 were obtained during a period in which the patient was receiving 35 units of protamine insulin every morning one half hour before breakfast. When this meal contained 39 grams (Curve 1) or 31 grams (Curve 3) of carbohydrate the glycemic curve was distinctly diabetic, but when only 19 grams of carbohydrate were given (Curve 2) the curve was normal Curves 4 and 5 were obtained after the time of administration of the protamine insulin had been changed from morning to evening Although 35 units kept the blood sugar fairly constant at an even subnormal level without food during the next morning the noon meal containing 25 grams of carbohydrate resulted in an unduly prolonged elevation (Curve 5) and on another day despite a hypoglycemic start, the curve after the usual breakfast containing 39 grams of carbohydrate was abnormal (Curve 4)

Moderately severe to severe diabetes

Patient B S (Figure 3) With the patient receiving 50 units of protamine insulin every morning including the morning of the first test, a breakfast containing only

Patient J R., No 176278. Severity Mild. Sex M. Age 61 Duration 3 yrs.



Curve 1 7 29 37 After usual breakfast, 33 gm. CHO 15 u. P.I given preceding morning

Curve 2. 8-3-37 No food. 15 u. P.I given preced ing morning

Curve 3. 8-5 37 After 33 gm. glucose. 20 u. P.I. given preceding morning

Complications and Course Early June, 1937 treated for ameblasis with emetine and yatren. July 6, 1937, combined abdomino-perineal resection of rectum for carc. Good recovery No fever for 11 days before first test. No glycosuria.

Diet C 100 P 59 F 129

Insulin 15 u. protamine zinc insulin ½ hour before breakfast every morning except on test days, when same dose was given at conclusion of test. The test with glu cose was preceded by 20 u. given previous morning Eventual requirement 20 u. P I. daily

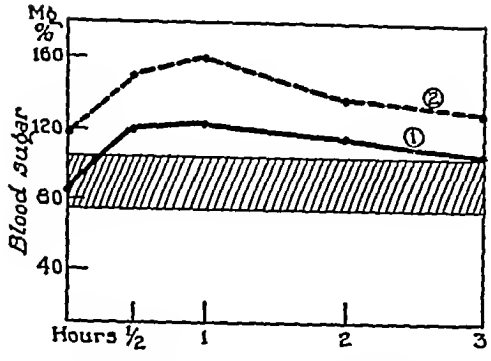
FIG 4 BLOOD SUGAR CURVES AFTER PROTAMINE INSULIN IN A DIABETIC PATIENT WITH AND WITHOUT FOOD

24 grams of carbohydrate still resulted in a blood sugar considerably above normal at the 2 hour period and slightly above at 3 hours (Curve 1) It was through an error that any insulin was given on this morning and this coupled with the fact that it had been neces sary to reduce the carbohydrate of the breakfast from 40 grams to 24 grams in order to control glycosuria with protamine insulin alone, may account for the curves not being more "diabetic" than it is. Curve 2 demon strates the ability of 50 units of protamine insulin given 24 hours earlier to maintain the blood sugar at a normal level without food.

Mild diabetes

Patient J R (Figure 4) All curves were obtained during a period in which the patient was receiving an injection of protamine insulin each morning before

Patient F M, No 149886 Severity Mild. Age 69 Sex F Duration 5 years



Curve 1 7-20-37 After usual breakfast, 33 gm. CHO 10 u P I given preceding morning

Curve 2 7-26-37 After usual breakfast, 33 gm. CHO No insulin for 4 days

Complications Nephrosclerosis, hypertension, generalized arteriosclerosis, diabetic and hypertensive retinitis

Course F B S 1936 = 211 mg % Sugar free throughout. No hypoglycemia

Diet C 100, P 75, F 80

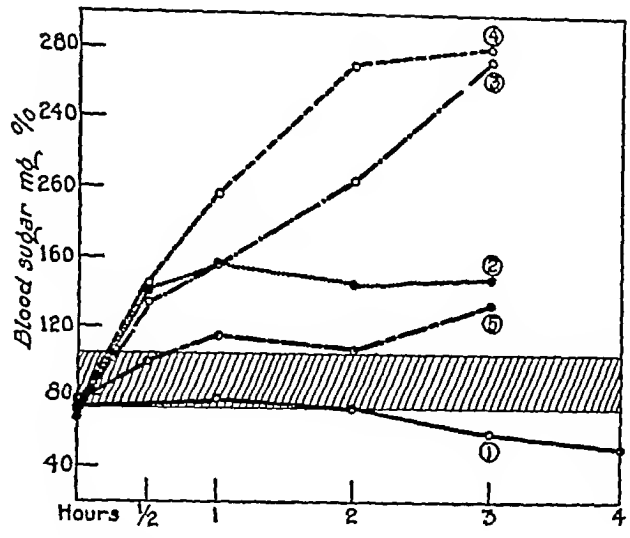
Insulin 10 u P I ½ hr before breakfast each morning except first test day All insulin stopped July 22

FIG 5 BLOOD SUGAR CURVES AFTER PROTAMINE INSULIN IN A DIABETIC PATIENT WITH AND WITHOUT FOOD

breakfast. No insulin, however, was given on any test morning, the dose for that day being given just before lunch and after the conclusion of the test. Curve 1 shows that 15 units of protamine insulin administered 24 hours earlier rendered the fasting blood sugar practically normal but was inadequate to effect a normal response after a breakfast containing 33 grams of carbohydrate. It is to be noted, however, that this curve is not as abnormal as the curves seen in the cases of severe diabetes (Figures 1 and 2) after a similar meal That the dose of 15 units was a little short of being optimum is shown by the gradual rise of Curve 2, which was obtained under identical conditions except that breakfast was withheld. When the test meal consisted of glucose (Curve 3) in an amount equal to the carbohydrate of the patient's usual breakfast (33 grams), the sugar tolerance curve was even more abnormal despite the larger dose of protamine insulin (20 units) administered the preceding morning

Patient F M (Figure 5) With the patient receiving a daily injection of protamine insulin, her usual breakfast, containing 33 grams of carbohydrate, resulted in a practically normal blood sugar curve (Curve 1) The mildness of her disease is shown by Curve 2, obtained after a similar breakfast but 4 days after withdrawal of all insulin. The fact that the patient was nevertheless diabetic is indicated by the fasting blood sugar of 211 mgm. per cent in 1936

Dog A—Diabetic. Sex M Wt 7 5 kg Depancreatized 12-8-37



First Experiment 1-11-38 Wound healed and dog in good condition.

Each blood sugar curve obtained in the morning after a preceding evening dose of 5 u P I

① No food.
② After usual breakfast, 25 gm CHO
③ Same
④ After usual breakfast, 12 gm CHO
⑤ Breakfast but no CHO

Diet Two daily feedings, 9 A.M and 6 P M, each consisting of 150 gm. ground beef heart, 50 gm. raw beef pancreas, 25 gm. cane sugar, 7 gm. cod liver oil, ± 4 gm. NaCl

Insulin 6 u. before each feeding

Glycosuria 0 to ++

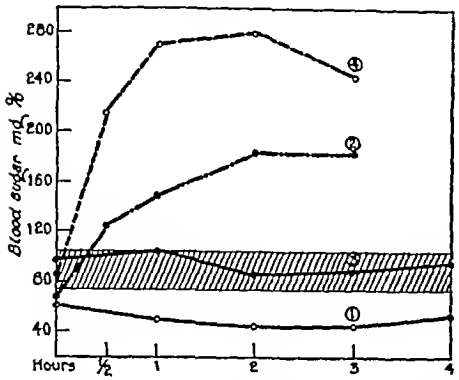
FIG 6 BLOOD SUGAR CURVES AFTER PROTAMINE INSULIN IN A DEPANCREATIZED DOG WITH AND WITHOUT FOOD

Totally depancreatized dogs[3]

Dog A (Figure 6) The first of these experiments was performed over a month after complete pancreatectomy when the dog was in good condition and the wound well healed The animal had maintained its weight and exhibited a moderate glycosuria while receiving two daily feedings as indicated in the chart with 6 units of regular insulin before each feeding This schedule was adhered to throughout except on the test days, which were never less than two days apart and usually longer The evening before each experiment 5 units of protamine insulin were given subcutaneously, and no insulin was given on the morning of the test. Curve 1 demonstrates that this dose held the blood sugar normal or below during the entire morning without food.

[3] The author is indebted to Dr Carter Goodpasture for performing the pancreatectomies

Dog B—Diabetic. Sex M. Wt. 10.5 kg Depancreatized 12-8-37



First Experiment, 1 28-38 Wound practically healed at this time, completely healed before next experiment. Dog in good condition.

Curve ① No food. 3 u. R + 6 u. P.I. given preceding evening

Curve ② After usual breakfast, 25 gm. CHO In sulin as above.

Curve ③ No food. 3 u. R + 4 u. P.I. given preceding evening

Curve ④ After usual breakfast, 25 gm. CHO In sulin as above.

Diet Two daily feedings, 9 A.M and 6 P.M., each consisting of 200 gm. ground beef heart, 50 gm. raw beef pancreas, 25 gm. cane sugar, 7 gm. cod liver oil ± 4 gm. NaCl.

Insulin 15 u. before each feeding.

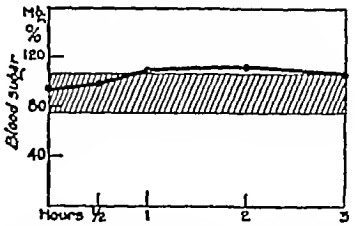
Glycosuria 0 to

FIG. 7 BLOOD SUGAR CURVES AFTER PROTAMINE INSULIN IN A DEPANCREATIZED DOG WITH AND WITHOUT FOOD

Curves 2 and 3 obtained after the animal s usual breakfast containing 25 grams of cane sugar and Curve 4 preceded by a breakfast containing only 12 grams of sugar are all definitely diabetic. The facts that the same amount of sugar in the diet gave different types of curves on different days and that 12 grams of sugar resulted in greater hyperglycemia than 25 grams are probably to be explained by differences in absorption. The slight though prolonged rise of Curve 5 illustrates the improved control of the blood sugar by protamine insulin when the source of the dietary carbohydrate is protein instead of preformed carbohydrate.

Five weeks after the last experiment this dog died suddenly in convulsions Fatty infiltration of the liver was slight in three out of four histological sections but

Dog X—Normal. Wt. 10.7 kg



Behavior of blood sugar curve of normal dog fed for two days on diet identical with that for Dog B

FIG. 8. BLOOD SUGAR CURVE IN A NORMAL DOG WITH FOOD

was marked in two small areas in the last. No pancreatic tissue was found.

Dog B (Figure 7) The experimental procedure was essentially the same as that for Dog A. Curves 1 and 2 show the blood sugar response without and with food respectively after the animal had been given 3 units of regular insulin and 6 units of protamine insulin the evening before each experiment. Since this dose led to hypoglycemia, the observations were repeated using 4 instead of 6 units of protamine insulin (Curves 3 and 4) Both curves obtained after food are clearly diabetic even though Curve 2 starts at a subnormal level.

Normal dog

Dog X (Figure 8) Dog X was fed for two days on a diet identical with that used for Dog B The blood sugar curve obtained after breakfast on the third morning gives no evidence that such a feeding is more than a normal animal of similar weight could be expected to handle adequately

COMMENT

It is apparent that in severely diabetic patients and totally diabetic (depancreatized) dogs a dose of protamine insulin capable of maintaining a normoglycemic plateau without food is not adequate to control the blood sugar after a moderate carbohydrate meal In other words, postprandial hyperglycemia in severe diabetes is not prevented without extra insulin On the other hand, under similar conditions the milder the diabetes the more nearly normal is the blood sugar curve. This suggests that the pancreas of the mild diabetic is better able to supply extra insulin at the time it is needed and, further, that the pancreas

of the normal individual is completely able to do so

These concepts are in harmony with the theory that, in health, the secretion of insulin is regulated, either directly or through the mediation of the nervous system, by the height of the blood sugar This theory has definite experimental support Houssay, Lewis, and Foglia (5) demonstrated that the blood sugar of a normal dog is not altered if as many as three extra pancreases are grafted into its neck, indicating that the secretion of each is depressed and regulated according to the level of the blood sugar LaBarre (6), Zunz and LaBarre (7), London and Kotschneff (8), Barbas and Schulutko (9), and others have shown that hyperglycemia induced in one animal stimulates the formation of a substance, presumably insulin, obtained from the pancreaticoduodenal vein which, when injected into another animal, lowers the blood sugar of the recipient Zunz and LaBarre have also shown that hypoglycemia produced in an animal by insulin (10), inhibits the secretion of insulin by that animal

It may be contended that the arguments presented in this paper that normally there is a pancreatic increatory response to hyperglycemia are based on the hypothesis that diabetes is purely or largely pancreatic in origin and that this theory, in view of the rôle now known to be played by the anterior pituitary, is no longer tenable It must be pointed out that, whatever the pathogenesis of diabetes, it always exists as a *relative* insulin deficiency, for the disturbed metabolism which characterizes it can be restored to normal by insulin

The conclusion that additional insulin is required to bring about a normal return of the blood sugar after a meal is at variance with that reached by Soskin, Allweiss, and Cohn (2) in their work with depancreatized dogs The difference in experimental results is probably to be explained by differences in experimental procedure It should be pointed out that what an animal can be made to do under artificial conditions and what it actually does as a matter of everyday existence are not necessarily the same thing The experiments of Soskin and his coworkers may fall in the former category It is possible that the constant injection of glucose and insulin given in order to maintain the blood sugar at a normal level for an hour before the administration of the test dose might so accelerate the metabolism of carbohydrate that the addition of more glucose would produce only a moderate disturbance of the equilibrium

Evidence for such a supposition is to be found in the tests performed in the case of Patient M S (Figure 9) In this case of moderately severe diabetes, as in other similar cases described above, a diabetic glucose tolerance curve was obtained after the fasting blood sugar had been brought to normal by protamine insulin given the preceding evening (Curve 1) Five days later, after a similar dose of protamine insulin given the previous evening, the patient received a continuous intravenous injection of glucose and insulin in such proportion as might be expected to maintain the blood sugar normal and level At the third hour an oral glucose tolerance test was performed, the intravenous injection being continued to the end of the experiment The resulting blood sugar curve (Curve 2) is essentially normal in shape, though, owing to a slight excess of insulin over glucose in the intravenous fluid, it begins and ends at subnormal levels While this experiment does not determine whether the nor-

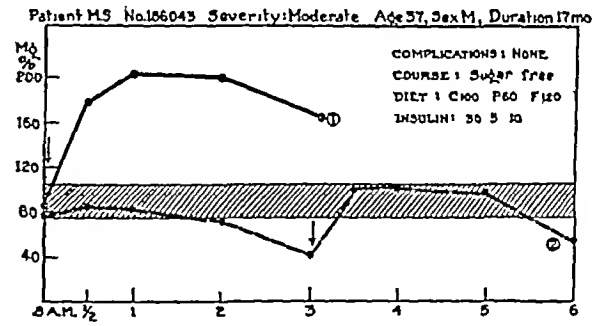


FIG 9 GLUCOSE TOLERANCE CURVES IN A DIABETIC SUBJECT (1) AFTER PROTAMINE INSULIN AND (2) DURING CONTINUOUS INTRAVENOUS INJECTION OF GLUCOSE AND INSULIN

Arrows indicate administration of 33 grams of glucose by mouth

Curve 1 25 units of protamine insulin and 5 units of regular insulin given before supper the previous evening

Curve 2 Protamine and regular insulin given the previous evening as before At 8 a.m a continuous intravenous infusion was begun, delivering 0.2 gram of glucose and 0 1 unit of regular insulin in 1 0 cc. of fluid per kilogram per hour The injection was maintained throughout the experiment.

mality of the second tolerance curve is caused by the effect of preliminary glucose and insulin or possibly by the effect of extra insulin alone, it demonstrates that the shape of the curve in a diabetic subject can be altered toward the normal by this technic. It is maintained that the use of protamine insulin alone in such experiments permits a closer approximation to the normal metabolic status and that the results so obtained, therefore, have greater physiological significance.

The studies here reported do not in any way detract from the importance of the concept, emphasized by Soskin, that the homeostatic mechanism of the liver plays a large part in the regulation of the blood sugar They indicate, however, that, normally the proper functioning of this mechanism, when it is presented with the added burden of ingested carbohydrate, depends upon the availability of extra insulin

SUMMARY AND CONCLUSIONS

1 It is shown that in severe diabetes, with the fasting blood sugar brought to normal by protamine insulin, postprandial hyperglycemia is not controlled without extra insulin

2 Under similar conditions the blood sugar curves of mild diabetics approach the normal.

3 These facts do not support the contention that the liver operates to reduce hyperglycemia without the aid of extra insulin. They do offer new evidence in favor of certain old theories namely

(*a*) That normally the ingestion of carbohydrate stimulates the secretion of insulin by the pancreas

(*b*) That the pancreas of the severe diabetic responds poorly to such a stimulus

(*c*) That the pancreas of the mild diabetic retains enough of its incretory function to react when so stimulated by secreting an additional, though still not optimum, amount of insulin.

(*d*) That the blood sugar curve of the normal individual may be regarded as the result of a completely adequate pancreatic response

BIBLIOGRAPHY

1 Houssay B A. Diabetes as a disturbance of endocrine regulation. Am. J M. Sc., 1937 193, 581

2. Soskin, S., Allweiss, M D., and Cohn D J., Influence of the pancreas and the liver upon the dextrose tolerance curve. Am. J Physiol. 1934 109 155

3 Ricketts H. T., Problems connected with the use of protamine-zinc insulin. Ann. Int. Med., 1937 11, 777

4 Miller B F., and Van Slyke, D D., A direct microtitration method for blood sugar J Biol Chem., 1936, 114, 583

5 Houssay B A., Lewis, J T., and Foglia, V G., Action compensatrice ou préventive de la greffe pancréatique sur la glycémie diabétique ou normale. Compt. rend. Soc. de biol., 1929 100 140

Action de la greffe pancreatique sur les variations de la glycémie produites par l'injection de glucose. Ibid., 1929 100 142.

6 LaBarre, J., Les variations insulino-sécrétoires au cours des hyperglycemies extrapancréatiques. Compt. rend. Soc. de biol., 1931, 106 1247

7 Zunz, E., and LaBarre, J., Sur l'augmentation de la teneur en insuline du sang veineux pancréatique après l'hyperglycemie provoquée par injection de glucose. Compt. rend. Soc. de biol. 1927 96, 421

8. London, E. S., and Kotschneff N P., Inkretionsdynamik. II. Über einige physiologische Anreger der Inkretion der Bauchspeicheldrüse und der Nebennieren. Arch. f. d. ges Physiol., 1931 228, 533

9 Barbas M. J., and Schulutko J B., Die experimentelle inkretorische Analyse als Indicator der Schwere der diabetischen Affektion. Z. chr f. d. ges. exper Med., 1930, 95 729

10 Zunz, E., and LaBarre, J., L'hyperglycemie provoquée par injection d'insuline diminue-t-elle la production d'insuline par le pancréas? Compt. rend. Soc. de biol., 1927 96, 1045

T

I

J

K

EDITORIAL BOARD

RANDOLPH WEST, *Editor-in-Chief*

FRANCIS G BLAKE
HERRMAN L BLUMGART
JAMES L GAMBLE
ROBERT F LOEB
WILLIAM S MCCANN
C PHILLIP MILLER
JOHN P PETERS
THOMAS M RIVERS
FRANK N WILSON

EDITORIAL COMMITTEE

DAVID P BARR
WALTER BAUER
ARTHUR L BLOOMFIELD
ARLIE V BOCK
C SIDNEY BURWELL
THOMAS FRANCIS, JR.
GEORGE R HERRMANN
L EMMETT HOLT, JR
CHESTER S KEEFER
ROBERT F LOEB
EDWARD H MASON
HUGH J MORGAN
DAVID T SMITH
FRED M SMITH
ISAAC STARR

CONTENTS

Number 1, January, 1938

Number 2, March, 1938

NUMBER 3, MAY, 1938

Number 4, July, 1938

NUMBER 5, SEPTEMBER, 1938

Number 6, November, 1938